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                   alerts (SDIs) affected
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L5   ANSWER 1 OF 3  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2003274435  EMBASE  Angiogenesis inhibitors in genitourinary cancers. Stadler
     W.; Wilding G.. W. Stadler, Department of Medicine Surgery, Sections of
     Hematol., Oncol./Urology, University of Chicago, 5841 S. Maryland,
     Chicago, IL 60637, United States. wstadler@medicine.bsd.uchicago.edu.
     Critical Reviews in Oncology/Hematology  46/SUPPL. (S41-S47)  27 Jun 2003.
     Refs: 37.
     ISSN: 1040-8428. CODEN: CCRHEC. Pub. Country: Ireland. Language: English.
     Summary Language: English.
AB   Despite much enthusiasm, no clear clinical benefit to any antiangiogenic
     agent has yet been demonstrated. Phase I trials demonstrate that
     endostatin, an endothelial cell toxin, can be administered safely, but no
     obvious anti-tumor effects were observed. Certain matrix metalloproteinase
     inhibitors appear ineffective, but later generation inhibitors with less
     systemic toxicity continue to be investigated. There are occasional
     responses to thalidomide either singly or in combination, but its
     pharmacology and mechanism of action remain unclear. A randomized study
     with the anti-VEGF antibody bevacizumab suggests that
     VEGF pathway is an important target in renal cell cancer. VEGF
     receptor tyrosine kinase inhibitors continue to be developed, but
     one of the first compounds SU5416 has had minimal clinical effects.
     Clinical trial designs that address the stable disease endpoint should
     thus be investigated and the randomized discontinuation design has already
     been tested. Pharmacodynamic markers that reflect antiangiogenic drug
     effect also need to be developed, but the putative ones, including
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circulating proangiogenic factors, tumor microvessel density, and dynamic contrast MRI have not yet proven to be useful. .COPYRGT. 2003 Elsevier Science Ireland Ltd. All rights reserved.

L5 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
2002:444883 Document No. 137:150608 Vascular endothelial growth factor (VEGF)-induced angiogenesis in herniated disc resorption. Haro, Hirotaka; Kato, Tsuyoshi; Komori, Hiromichi; Osada, Motonobu; Shinomiya, Kenichi (Division of Locomotorial Molecular Degeneration Research, Human Genes and Science Center, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, 113-5819, Japan). Journal of Orthopaedic Research, 20(3), 409-415 (English) 2002. CODEN: JOREDR. ISSN: 0736-0266. Publisher: Elsevier Science Ltd..

AB Intervertebral disk herniation is a major cause of low back pain and sciatica. Spontaneous resorption of herniated disk (HD) is frequently detected by magnetic resonance **imaging** (MRI). Marked infiltration by macrophages and neo-vascularization are observed upon histogical examination of HD. In addition, enhanced MRI studies suggest that HD resorption occurs more frequently in those completely exposed to the epidural space and that this correlates with their degree of vascularization. We have postulated that the angiogenic factor, vascular endothelial growth factor (VEGF), may be implicated in the neo-vascularization of HD tissues. Here we demonstrate that VEGF and its receptors VEGFR-1 and VEGFR-2 are expressed in human surgical samples of HD. Using a co-culture system comprised of murine peritoneal macrophages and intervertebral disk tissue as a model of the acute phase of HD developed previously, an increase in macrophage VEGF protein and mRNA expression was observed upon exposure to disk tissue. Tumor necrosis factor alpha (TNF-α) was required for this induction of VEGF. Use of a novel angiogenesis assay revealed that addition of the conditioned media from the co-culture system resulted in an increase of vascular tubule formation. This effect was strongly inhibited by anti-**VEGF antibody**, but augmented by recombinant VEGF. We conclude that VEGF induction, under the co-culture conditions tested can result in neo-vascularization of intervertebral disk tissue and may thus play a role in the resorption of HD.

L5 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
2000:772677 Document No. 133:349140 Compositions and methods for cancer treatment by selectively inhibiting VEGF. Thorpe, Philip E.; Brekken, Rolf A. (Board of Regents, the University of Texas System, USA). PCT Int. Appl. WO 2000064946 A2 20001102, 297 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US11367 20000428. PRIORITY: US 1999-PV131432 19990428.

AB Disclosed are antibodies that specifically inhibit VEGF binding to only one (VEGFR2) of the two **VEGF receptors.** The antibodies effectively inhibit angiogenesis and induce tumor regression, and yet have improved safety due to their specificity. The present invention thus provides new antibody-based compns., methods and combined protocols for treating cancer and other angiogenic diseases. Advantageous immunoconjugate and prodrug compns. and methods using the new VEGF-specific antibodies are also provided.

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L6 28 DUP REMOVE L2 (37 DUPLICATES REMOVED)

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L6 ANSWER 1 OF 28 MEDLINE on STN DUPLICATE 1
2004219635. PubMed ID: 15078792. Aldosterone enhances ischemia-induced neovascularization through angiotensin II-dependent pathway. Michel Frederic; Ambroisine Marie-Lory; Duriez Micheline; Delcayre Claude; Levy Bernard I; Silvestre Jean-Sebastien. (INSERM U541, Hopital Lariboisiere, IFR Circulation-Lariboisiere, Universite Paris, Paris, France.) Circulation, (2004 Apr 27) 109 (16) 1933-7. Journal code: 0147763. ISSN: 1524-4539. Pub. country: United States. Language: English.

AB BACKGROUND: We analyzed the role of aldosterone in ischemia-induced neovascularization and the involvement of angiotensin II (Ang II) signaling in this effect. METHODS AND RESULTS: Ischemia was induced by right femoral artery ligature in mice treated or not with aldosterone (4.5 microg/day), aldosterone plus spironolactone (aldosterone receptor blocker; 20 mg/kg per day), or aldosterone plus valsartan (angiotensin type 1 [AT1] receptor blocker; 20 mg/kg per day). After 21 days, neovascularization was evaluated by microangiography, capillary density measurement, and laser-Doppler perfusion **imaging.** Protein level of vascular endothelial growth factor (VEGF) was determined by Western blot analysis in hindlimbs. mRNA levels of renin-angiotensin system components were also assessed by semiquantitative reverse transcription-polymerase chain reaction. Angiographic score, capillary number, and foot perfusion were improved in ischemic/nonischemic leg ratio by 1.4-, 1.5-, and 1.4-fold, respectively, in aldosterone-treated mice compared with controls ($P<0.05$). Aldosterone proangiogenic effect was associated with 2.3-fold increase in VEGF protein content ($P<0.05$). Treatments with spironolactone or with neutralizing **VEGF antibody** hampered the proangiogenic effect of aldosterone ($P<0.05$ versus aldosterone-treated mice). Interestingly, AT1 receptor blockade completely abrogated the aldosterone proangiogenic effect, emphasizing the involvement of Ang II-related pathway in aldosterone-induced vessel growth. In this view, angiotensinogen mRNA content was 2.2-fold increased in aldosterone-treated mice in reference to controls ($P<0.05$), whereas that of renin, angiotensin-converting enzyme, and AT1 receptor subtype was unaffected. Aldosterone treatment also decreased AT2 mRNA content by 2-fold ($P<0.05$ versus controls), suggesting that aldosterone may switch the Ang II pathway toward activation of vessel growth. CONCLUSIONS: This study shows for the first time that aldosterone increases neovascularization in the setting of ischemia through activation of Ang II signaling.

L6 ANSWER 2 OF 28 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 2
2004460623 EMBASE MRI monitoring of Avastin® antiangiogenesis therapy using B22956/1, a new blood pool contrast agent, in an experimental model of human cancer. Preda A.; Novikov V.; Moglich M.; Turetschek K.; Shames D.M.; Brasch R.C.; Cavagna F.M.; Roberts T.P.L.. Dr. T.P.L. Roberts, Dept. of Medical Imaging, University of Toronto, 150 College St., Toronto, Ont. M5S 3E2, United States. Tim.Roberts@utoronto.ca. Journal of Magnetic Resonance Imaging 20/5 (865-873) 2004.
Refs: 47.
ISSN: 1053-1807. CODEN: JMRIFR. Pub. Country: United States. Language: English. Summary Language: English.

AB Purpose: To evaluate the diagnostic and prognostic potential of a new protein-binding contrast medium, B22956/1, for quantitatively characterizing tumor microvessels by MRI and monitoring response to antiangiogenic therapy. Materials and Methods: Dynamic contrast-enhanced MRI (DCE-MRI) was performed in an experimental cancer model with the use of the novel protein-binding agent B22956/1. a low molecular contrast agent (ProHance®), and a macromolecular contrast medium. albumin-(Gd-DTPA). MDA-MB-435, a human cancer cell line, was implanted in 22 athymic rats. Animals were assigned randomly to a control (saline) or drug-treated (Avastin®) group. MRI was performed at baseline and after

nine days of treatment. The transendothelial permeability (K (PS)) and the fractional blood volume (fBV) were estimated from the kinetic analysis of dynamic MR data using a two-compartment model. Tumor growth was also measured from volumetric MRI. Results: Tumors grew more slowly, although not significantly ($P = 0.07$), in the drug-treated group. The K (PS) determined for B22956/1 decreased significantly in the drug-treated group compared to baseline ($P < 0.05$). and progressed significantly in the control group. However, no significant changes were resolved with the use of ProHance or albumin-(Gd-DTPA). Conclusion: With the use of appropriate contrast media, the therapeutic effects of an anti-**VEGF antibody** on tumor microvessels can be monitored by dynamic MRI. The dynamic range of permeability to B22956/1, and the sensitivity to change of this parameter suggest a potential application in the clinical setting.

L6 ANSWER 3 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:427114 Document No.: PREV200400426987. Rat liver sinusoidal endothelial cell phenotype is maintained by paracrine and autocrine regulation. DeLeve, Laurie D. [Reprint Author]; Wang, Xiangdong; Hu, Liping; McCuskey, Margaret K.; McCuskey, Robert S.. Keck Sch MedDiv Gastrointestinal and Liver Dis, Univ So Calif, 2011 Zonal Ave HMR 603, Los Angeles, CA, 90033, USA. deleve@usc.edu. American Journal of Physiology - Gastrointestinal and Liver Physiology, (October 2004) Vol. 287, No. 4, pp. G757-G763. print. ISSN: 0193-1857 (ISSN print). Language: English.

AB The phenotypic features of liver sinusoidal endothelial cells (SEC), open fenestrae in sieve plates and lack of a basement membrane, are lost with capillarization. The current study examines localization of CD31 as a marker for the dedifferentiated, nonfenestrated SEC and examines regulation of SEC phenotype in vitro. CD31 localization in SEC was examined by confocal microscopy and immunogold-scanning electron microscopy. SEC cultured for 1 day express CD31 in the cytoplasm, whereas after 3 days, CD31 is also expressed on cell-cell junctions. Immunogold-scanning electron microscopy confirmed the absence of CD31 surface expression on fenestrated SEC 1 day after isolation and demonstrated the appearance of CD31 surface expression on SEC that had lost fenestration after 3 days in culture. SEC isolated from fibrotic liver do show increased expression of CD31 on the cell surface. Coculture with either hepatocytes or stellate cells prevents CD31 surface expression, and this effect does not require heterotypic contact. The paracrine effect of hepatocytes or stellate cells on SEC phenotype is abolished with anti-**VEGF antibody** and is reproduced by addition of VEGF to SEC cultured alone. VEGF stimulates SEC production of nitric oxide. NG-nitro-L-arginine methyl ester blocked the paracrine effect of hepatocytes or stellate cells on SEC phenotype and blocked the ability of VEGF to preserve the phenotype of SEC cultured alone. In conclusion, surface expression of CD31 is a marker of a dedifferentiated, nonfenestrated SEC. The VEGF-mediated paracrine effect of hepatocytes or stellate cells on maintenance of SEC phenotype requires autocrine production of nitric oxide by SEC.

L6 ANSWER 4 OF 28 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 3

2004527052 EMBASE Decrease in tumor apparent permeability-surface area product to a MRI macromolecular contrast medium following angiogenesis inhibition with correlations to cytotoxic drug accumulation. Daldrup-Link H.E.; Okuhata Y.; Wolfe A.; Srivastav S.; Oie S.; Ferrara N.; Cohen R.L.; Shames D.M.; Brasch R.C.. Dr. R.C. Brasch, Department of Radiology, Univ. of California San Francisco, 521 Parnassus Ave., San Francisco, CA 94143-0628, United States. robert.brasch@radiology.ucsf.edu. Microcirculation 11/5 (387-396) 2004.
Refs: 25.
ISSN: 1073-9688. CODEN: MROCER. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Background: New strategies for cancer therapy include the combination of

angiogenesis inhibitors with cytotoxins. However, angiogenesis inhibitors may alter tumor microvessel structure and transendothelial permeability thereby reducing tumoral delivery of cytotoxic agents. The aim of this study was to estimate quantitatively the apparent permeability-surface area product (K(PS)) in tumors to a macromolecular contrast medium (MMCM), to follow changes in K(PS) induced by antibodies to vascular endothelial growth factor (anti-VEGF), and to correlate the findings with tumor accumulation of cisplatin, a highly protein-bound cytotoxin, and 5-fluorouracil (5-FU), a small unbound cytotoxin. Methods: Dynamic MRI enhanced with a MMCM (albumin-(Gd-DTPA (30)) was analyzed using a two-compartment tumor tissue model (plasma and interstitial water) to quantitatively estimate K(PS). These estimates of K(PS) were correlated with cytotoxic drug accumulations in the tumors. Results: Anti-VEGF treatment reduced K(PS) to MMCM in tumor tissue from 0.013 mL h(-1) cm(-3) (n = 9) at baseline to 0.003 mL h(-1) cm(-3) (n = 9) 24 h later ($p < .05$). The K(PS) values correlated significantly ($r(2) = .78$; $p < .0001$) with the tumor cisplatin accumulation. No correlation ($r(2) = .001$; $p = .89$) was found between K(PS) and tumor accumulation of the substantially smaller 5-FU molecule. Conclusions: MMCM-enhanced MRI can be used to detect and estimate changes in K(PS) to this contrast agent following a single dose of anti-**VEGF antibody.** The decline in K(PS) induced by this inhibitor of angiogenesis is associated with reduced tumor concentration of a protein-bound cytotoxin, similar in molecular weight to the contrast agent. MRI assays of microvascular status as performed here may be useful to clinically monitor responses to anti-angiogenesis drugs and to optimize the choice and timing of cytotoxic drug administration. Copyright .COPYRGT. 2004 Taylor & Francis Inc.

L6 ANSWER 5 OF 28 MEDLINE on STN DUPLICATE 4

2004346213. PubMed ID: 15249461. Respiratory syncytial virus causes increased bronchial epithelial permeability. Kilani Muna M; Mohammed Kamal A; Nasreen Najmunnisa; Hardwick Joyce A; Kaplan Mark H; Tepper Robert S; Antony Veena B. (Indiana University Medical Center, Indianapolis, USA.) Chest, (2004 Jul) 126 (1) 186-91. Journal code: 0231335. ISSN: 0012-3692. Pub. country: United States. Language: English.

AB BACKGROUND: Respiratory syncytial virus (RSV)-induced diseases are mediated through active cytokines released during infection. We hypothesized that RSV infection causes bronchial epithelial monolayer permeability in vitro via induction of vascular endothelial growth factor (VEGF). METHODS: Human bronchial epithelial cells were infected with RSV. In some cultures, **VEGF antibody** was included to block VEGF response; in other cultures, palivizumab was added to block RSV infection. Permeability was assessed in real-time using electric cell-substrate impedance sensing. VEGF release was assessed using enzyme-linked immunosorbent assay. Gap formation was assessed using live cell **imaging.** RESULTS: RSV-infected cells demonstrated a decrease in the resistance of the monolayer indicating an increase in permeability; this increase was blocked with VEGF-specific antibody, and palivizumab. Intercellular gap formation developed in RSV-infected epithelial monolayers. CONCLUSION: RSV increases permeability of the bronchial airway epithelial monolayer via VEGF induction.

L6 ANSWER 6 OF 28 MEDLINE on STN DUPLICATE 5

2004319251. PubMed ID: 15221819. Tumor microvascular changes in antiangiogenic treatment: assessment by magnetic resonance contrast media of different molecular weights. Turetschek Karl; Preda Anda; Novikov Viktor; Brasch Robert C; Weinmann Hanns J; Wunderbaldinger Patrick; Roberts Timothy P L. (Center for Pharmaceutical and Molecular Imaging, Department of Radiology, University of California, San Francisco, California, USA.) Journal of magnetic resonance imaging : JMRI, (2004 Jul) 20 (1) 138-44. Journal code: 9105850. ISSN: 1053-1807. Pub. country: United States. Language: English.

AB PURPOSE: To test magnetic resonance (MR) contrast media of different molecular weights (MWs) for their potential to characterize noninvasively microvascular changes in an experimental tumor treatment model. MATERIALS

AND METHODS: MD-MBA-435, a poorly differentiated human breast cancer cell line, was implanted into 31 female homozygous athymic rats. Animals were assigned randomly to a control (saline) or drug treatment (monoclonal antibody vascular endothelial growth factor (Mab-**VEGF**) **antibody**) group. In both groups, dynamic MR **imaging** (MRI) was performed in each animal using up to three different contrast media on sequential days at baseline and follow-up examination. The MWs of the contrast media used ranged from 557 Da to 92 kDa. Using a bidirectional kinetic model, tumor microvessel characteristics, including the fractional plasma volume (fPV) and transendothelial permeability (K(PS)), were estimated for each contrast medium. These microvascular characteristics were compared between drug and control groups and between contrast media of different MWs. RESULTS: Tumors grew significantly slower ($P < 0.0005$) in the drug treatment group than in the control group. Mean K(PS) and fPV values decreased significantly ($P < 0.05$) in the Mab-**VEGF antibody**-treated group compared to baseline values using intermediate or macromolecular contrast media (MMCM), but did not change significantly using small molecular contrast media (SMCM). In the control groups, mean K(PS) and mean fPV values did not reach statistical significance for any of the contrast media used. CONCLUSION: Therapeutic effects of a Mab-**VEGF antibody** on tumor microvessel characteristics can be monitored by dynamic MRI. Intermediate-size agents, such as Gadomer-17, offer a substantial dynamic range and are less limited by **imaging** precision and therefore should be considered a practical alternative to monitor antiangiogenesis treatment effects in a clinical setting.
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L6 ANSWER 7 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:562510 Document No.: PREV200300551171. Angiogenesis induced by endothelial nitric oxide synthase gene through vascular endothelial growth factor expression in a rat hindlimb ischemia model. Namba, Tsunetatsu; Koike, Hiromi; Murakami, Kazushi; Aoki, Motokuni; Makino, Hirofumi; Hashiya, Naotaka; Ogihara, Toshio; Kaneda, Yasufumi; Kohno, Masakazu; Morishita, Ryuichi [Reprint Author]. Division of Clinical Gene Therapy, Medical School, Osaka University, 2-2 Yamada-oka, Suita, 565-0871, Japan. morishit@cgt.med.osaka-u.ac.jp. Circulation, (November 4 2003) Vol. 108, No. 18, pp. 2250-2257. print.
ISSN: 0009-7322 (ISSN print). Language: English.

AB Background: Because the mechanism of the angiogenic property of nitric oxide (NO) was not fully understood in vivo, we focused on the role of vascular endothelial growth factor (VEGF) in angiogenesis induced by endothelial NO synthase (eNOS) gene transfer. Methods and Results: After intramuscular injection of eNOS DNA into a rat ischemic hindlimb, transfection of eNOS vector resulted in a significant increase in eNOS protein 1 week after transfection. In addition, tissue concentrations of nitrite and nitrate were significantly increased in rats transfected with the eNOS gene up to 2 weeks after transfection. The increase in tissue nitrite and nitrate concentrations was completely inhibited by NG-nitro-L-arginine methyl ester (L-NAME). In contrast, serum concentrations of nitrite and nitrate and blood pressure were not changed by eNOS gene transfer. Importantly, overexpression of the eNOS gene resulted in a significant increase in peripheral blood flow, whereas L-NAME inhibited the increase in blood flow. Interestingly, basal blood flow was significantly lower in rats treated with L-NAME than in control rats. A significant increase in capillary number was consistently detected in rats transfected with the eNOS gene at 4 weeks after transfection, accompanied by a significant increase in VEGF. Moreover, administration of neutralizing anti-**VEGF antibody** abolished the increase in blood flow and capillary density induced by eNOS plasmid injection. Conclusions: Overall, intramuscular injection of bovine eNOS plasmid induced therapeutic angiogenesis in a rat ischemic hindlimb model, a potential therapy for peripheral arterial disease. The stimulation of angiogenesis by NO might be due to upregulation of local

VEGF expression.

L6 ANSWER 8 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:464776 Document No.: PREV200400463305. Vascular endothelial growth factor expression in the rat dural arteriovenous fistula model. Shin, Yasushi [Reprint Author]; Uranishi, Ryunosuke; Nakase, Hiroyuki; Sakaki, Toshisuke. Dept Neurosurg, Nara Med Univ, 840 Shijo Machi,Kashihara, Nara, 6348522, Japan. Brain and Nerve (Tokyo), (November 2003) Vol. 55, No. 11, pp. 946-952. print.
CODEN: NOTOA6. ISSN: 0006-8969. Language: Japanese.

AB Although various mechanisms of the development of dural arteriovenous fistula (DAVF) have been proposed, the pathogenesis of these lesions are still unclear. Recent experimental evidence suggested a role of angiogenic growth factors in the genesis of vascular malformations of the central nervous system. To further investigate the pathogenesis of DAVF, we examined the expression of the angiogenic growth factor, vascular endothelial growth factor (VEGF), in rat DAVF model. Material and Methods : Male Wistar rats (weighting 280 to 300 g, n=40) were used. Each rat was mounted on a stereotaxic frame under general anesthesia. DAVF model (Spetzler et al.) was made by common carotid artery-external jugular vein anastomosis, bipolar coagulation of the vein draining the transverse sinus, and superior sagittal sinus thrombosis (SSS). SSS was thrombosed by a hemostatic agent through 16-gauge needle. Venous hypertension was induced in 30 rats, which were divided into two experimental groups; (1) immunohistological study group (n=15) and (2) angiography group (n=15). Immunohistological analysis was performed by **VEGF antibody** 1 week after, and angiography was done 90 days after the surgery. Developing of angiographical DAVF was observed with the magnifying X-ray camera. Each 5 rats served as shamoperated controls, which received a similar surgery without induction of venous hypertension. Results : VEGF expression and DAVF were not observed in sham group. In immunohistological study group, VEGF expression in the endothelium and the connective tissues of the dura mater in the five rats (33%) and in the neurons in the eleven rats (73%) of the cerebral cortex and the basal ganglia were identified. In angiography group, DAVF formed in 6 among 15 rats (40%). Conclusion : The findings of this study provide the first experimental evidence that angiogenic growth factors VEGF may participate in the genesis of DAVF, These results suggest a novel strategy for the management and prevention of DAVIT and related disorders.

L6 ANSWER 9 OF 28 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003512585 EMBASE The quest for surrogate markers of angiogenesis: A paradigm for translational research in tumor angiogenesis and anti-angiogenesis trials. Ruegg C.; Meuwly J.-Y.; Driscoll R.; Werffeli P.; Zaman K.; Stupp R.. C. Ruegg, Laboratory of the CePO, ISREC, 155 Chemin des Boveresses, CH-1066 Epalinges Laussane, Switzerland. curzio.ruegg@isrec.unil.ch.
Current Molecular Medicine 3/8 (673-691) 2003.
Refs: 224.
ISSN: 1566-5240. CODEN: CMMUBP. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB Inhibition of tumor angiogenesis suppresses tumor growth and metastatic spreading in many experimental models, suggesting that anti-angiogenic drugs may be used to treat human cancer. During the past decade more than eighty molecules that showed anti-angiogenic activity in preclinical studies were tested in clinical cancer trials, but most of them failed to demonstrate any measurable anti-tumor activity and none have been approved for clinical use. Recent results stemming from trials with anti-**VEGF antibodies**, used alone or in combination with chemotherapy, suggest that systemic anti-angiogenic therapy may indeed have a measurable impact on cancer progression and patient survival. From the clinical studies it became nevertheless clear that the classical endpoints used in anti-cancer trials do not bring sufficient discriminative power to monitor the effects of anti-angiogenic drugs. It

is therefore necessary to identify and validate molecular, cellular and functional surrogate markers of angiogenesis to monitor activity and efficacy of anti-angiogenic drugs in patients. Availability of such markers will be instrumental to re-evaluate the role of tumor angiogenesis in human cancer, to identify new molecular targets and drugs, and to improve planning, monitoring and interpretation of future studies. Future anti-angiogenesis trials integrating biological endpoints and surrogate markers or angiogenesis will require close collaboration between clinical investigators and laboratory-based researchers. Here we review and discuss critical issues and emerging paradigms relevant to tumor angiogenesis and anti-angiogenic drugs and to the monitoring of tumor angiogenesis and anti-angiogenic effects in patients.

L6 ANSWER 10 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:310687 Document No.: PREV200300310687. Vascular endothelial growth factor rescues 2,3,7,8-tetrachlorodibenzo-p-dioxin inhibition of coronary vasculogenesis. Ivnitski-Steele, I. D. [Reprint Author]; Walker, M. K. [Reprint Author]. University of New Mexico, Albuquerque, NM, USA. Birth Defects Research, (May 2003) Vol. 67, No. 5, pp. 324. print.
Meeting Info.: 43rd Annual Meeting of the Teratology Society. Philadelphia, PA, USA. June 21-26, 2003. Teratology Society.
ISSN: 1542-0752 (ISSN print). Language: English.

L6 ANSWER 11 OF 28 MEDLINE on STN DUPLICATE 6

2003253908. PubMed ID: 12778014. Renal cell carcinoma. Whang Young E; Godley Paul A. (Division of Hematology/Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7305, USA.) Current opinion in oncology, (2003 May) 15 (3) 213-6. Ref: 23. Journal code: 9007265. ISSN: 1040-8746. Pub. country: United States. Language: English.

AB Renal cell carcinoma (RCC) continues to present a diagnostic and therapeutic challenge. The increased use of abdominal **imaging** studies does not appear to completely account for the rising incidence of RCC. Alcohol consumption has been found to be a possible protective factor among women in a recent study, but among women with children, RCC risk may increase with each child born when compared with nulliparous women. An alternative staging system shows promise, and two randomized clinical trials clarify the role of removing the primary tumor in the setting of metastatic RCC. New agents have shown promise in early clinical trials such as CCI-779, pegylated interferon, thalidomide, and anti-**VEGF antibody.**

L6 ANSWER 12 OF 28 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003274435 EMBASE Angiogenesis inhibitors in genitourinary cancers. Stadler W.; Wilding G.. W. Stadler, Department of Medicine Surgery, Sections of Hematol., Oncol./Urology, University of Chicago, 5841 S. Maryland, Chicago, IL 60637, United States. wstadler@medicine.bsd.uchicago.edu. Critical Reviews in Oncology/Hematology 46/SUPPL. (S41-S47) 27 Jun 2003. Refs: 37.
ISSN: 1040-8428. CODEN: CCRHEC. Pub. Country: Ireland. Language: English. Summary Language: English.

AB Despite much enthusiasm, no clear clinical benefit to any antiangiogenic agent has yet been demonstrated. Phase I trials demonstrate that endostatin, an endothelial cell toxin, can be administered safely, but no obvious anti-tumor effects were observed. Certain matrix metalloproteinase inhibitors appear ineffective, but later generation inhibitors with less systemic toxicity continue to be investigated. There are occasional responses to thalidomide either singly or in combination, but its pharmacology and mechanism of action remain unclear. A randomized study with the anti-**VEGF antibody** bevacizumab suggests that VEGF pathway is an important target in renal cell cancer. VEGF receptor tyrosine kinase inhibitors continue to be developed, but one of the first compounds SU5416 has had minimal clinical effects. Clinical trial designs that address the stable disease endpoint should thus be investigated and

the randomized discontinuation design has already been tested. Pharmacodynamic markers that reflect antiangiogenic drug effect also need to be developed, but the putative ones, including circulating proangiogenic factors, tumor microvessel density, and dynamic contrast MRI have not yet proven to be useful. .COPYRGT. 2003 Elsevier Science Ireland Ltd. All rights reserved.

L6 ANSWER 13 OF 28 MEDLINE on STN DUPLICATE 7

2002498128. PubMed ID: 12359857. Molecular **imaging** and biological evaluation of HuMV833 anti-**VEGF antibody**: implications for trial design of antiangiogenic antibodies. Jayson Gordon C; Zweit Jamal; Jackson Alan; Mulatero Clive; Julyan Peter; Ranson Malcolm; Broughton Lynn; Wagstaff John; Hakannson Leif; Groenewegen Gerard; Bailey John; Smith Nigel; Hastings David; Lawrance Jeremy; Haroon Hamied; Ward Tim; McGown Alan T; Tang Meina; Levitt Dan; Marreaud Sandrine; Lehmann Frederic F; Herold Manfred; Zwierzina Heinz. (Cancer Research UK Department of Medical Oncology, Christie Hospital NHS Trust, Manchester, UK. (European Organisation for Research and Treatment of Cancer Biological Therapeutic Development Group). Gordon.Jayson@christie-tr.nwest.nhs.uk) . Journal of the National Cancer Institute, (2002 Oct 2) 94 (19) 1484-93. Journal code: 7503089. ISSN: 0027-8874. Pub. country: United States. Language: English.

AB BACKGROUND: Vascular endothelial growth factor (VEGF) is a potent angiogenic cytokine, and various inhibitory agents, including specific antibodies, have been developed to block VEGF-stimulated angiogenesis. We developed HuMV833, a humanized version of a mouse monoclonal anti-**VEGF antibody** (MV833) that has antitumor activity against a number of human tumor xenografts, and investigated the distribution and biologic effects of HuMV833 in patients in a phase I trial. METHODS: Twenty patients with progressive solid tumors were treated with various doses of HuMV833 (0.3, 1, 3, or 10 mg/kg). Positron emission tomography with (124)I-HuMV833 was used to measure the antibody distribution in and clearance from tissues. Magnetic resonance **imaging** was used to measure the vascular permeability surface area product with a first-pass pharmacokinetic model (k(fp)) to determine tumor vascular permeability. RESULTS: The antibody was generally well tolerated, although the incremental dose, phase I study design, and pharmacodynamic endpoints could not identify the optimum biologically active dose. Antibody distribution and clearance were markedly heterogeneous between and within patients and between and within individual tumors. HuMV833 distribution to normal tissues also varied among patients, but the antibody was cleared from these tissues in a homogeneous fashion. Permeability was strongly heterogeneous between and within patients and between and within individual tumors. All tumors showed a reduction in k(fp) 48 hours after the first treatment (median = 44%; range = 4%-91%). CONCLUSIONS: Because of the heterogeneity in tumor biology with respect to antibody uptake and clearance, we suggest that either intrapatient dose escalation approaches or larger, more precisely defined patient cohorts would be preferable to conventional strategies in the design of phase I studies with antiangiogenic compounds like HuMV833.

L6 ANSWER 14 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:36970 Document No.: PREV200300036970. Preparation of the iodine-124 derivative of the Bolton-Hunter reagent ((124I)I-SHPP) and its use for labelling a **VEGF antibody** as a PET tracer. Glaser, Matthias [Reprint Author]; Carroll, Veronica A.; Collingridge, David R.; Aboagye, Eric O.; Price, Pat; Bicknell, Roy; Harris, Adrian L.; Luthra, Sajinder K.; Brady, Frank. Imaging Research Solutions Ltd., Hammersmith Hospital, Du Cane Road, London, W12 ONN, UK. m.glaser@csc.mrc.ac.uk. Journal of Labelled Compounds and Radiopharmaceuticals, (October 30 2002) Vol. 45, No. 12, pp. 1077-1090. print.
ISSN: 0362-4803 (ISSN print). Language: English.

AB This study describes the radioiodination of an antibody specific to the vascular endothelial growth factor (VEGF), VG76e, with (124I)iodine to

obtain a novel PET tracer for measurement of angiogenesis. In vitro binding assays showed a significantly higher immunoreactive fraction with the protein labelling reagent N-succinimidyl 3-(4-hydroxy-5-(125I)iodophenyl) propionate ((125I)Bolton-Hunter reagent, (125I)I-SHPP) (34.0+-4.0%) as compared with N-succinimidyl 3-(125I)iodobenzoate (10.9+-6.4%) or direct radioiodination using (125I)iodide and IodoGen (3.1+-3.0%). Consequently, the cyclotron-produced positron-emitting (124I)iodine (T1/2=4.2 days) was employed to prepare (124I)I-SHPP. Using an improved radioiodination methodology, (124I)I-SHPP was prepared from sodium (124I)iodide with IodoGen at pH 6.5. The (124I)Bolton-Hunter reagent was isolated with 25-58% (n=3) radiochemical yield and 88-95% (n=3) radiochemical purity by the conventional extraction procedure. The conjugate of VG76e with (124I)I-SHPP was prepared with 17-18% (n=3) labelling efficiency and 98% radiochemical purity. The immunoreactive fraction was determined to be 33.5% (n=2).

L6 ANSWER 15 OF 28 MEDLINE on STN DUPLICATE 8

2002298442. PubMed ID: 12038611. Vascular endothelial growth factor (VEGF)-induced angiogenesis in herniated disc resorption. Haro Hirotaka; Kato Tsuyoshi; Komori Hiromichi; Osada Motonobu; Shinomiya Kenichi. (Division of Locomotorial Molecular Degeneration Research, Human Genes and Science Center, Tokyo Medical and Dental University, Graduate School of Medicine, Japan.. haro.orth@tmd.ac.jp) . Journal of orthopaedic research : official publication of the Orthopaedic Research Society, (2002 May) 20 (3) 409-15. Journal code: 8404726. ISSN: 0736-0266. Pub. country: United States. Language: English.

AB Intervertebral disc herniation is a major cause of low back pain and sciatica. Spontaneous resorption of herniated disc (HD) is frrequently detected by magnetic resonance **imaging** (MRI). Marked infiltration by macrophages and neo-vascularization are observed upon histogical examination of HD. In addition, enhanced MRI studies suggest that HD resorption occurs more frequently in those completely exposed to the epidural space and that this correlates with their degree of vascularization. We have postulated that the angiogenic factor, vascular endothelial growth factor (VEGF), may be implicated in the neo-vascularization of HD tissues. Here we demonstrate that VEGF and its receptors VEGFR-1 and VEGFR-2 are expressed in human surgical samples of HD. Using a co-culture system comprised of murine peritoneal macrophages and intervertebral disc tissue as a model of the acute phase of HD developed previously, an increase in macrophage VEGF protein and mRNA expression was observed upon exposure to disc tissue. Tumor necrosis factor alpha (TNF-alpha) was required for this induction of VEGF. Use of a novel angiogenesis assay revealed that addition of the conditioned media from the co-culture system resulted in an increase of vascular tubule formation. This effect was strongly inhibited by anti-**VEGF** **antibody**, but augmented by recombinant VEGF. We conclude that VEGF induction, under the co-culture conditions tested can result in neo-vascularization of intervertebral disc tissue and may thus play a role in the resorption of HD.

L6 ANSWER 16 OF 28 MEDLINE on STN DUPLICATE 9

2002159150. PubMed ID: 11891967. Dynamic contrast-enhanced magnetic resonance **imaging** as a surrogate marker of tumor response to anti-angiogenic therapy in a xenograft model of glioblastoma multiforme. Gossmann Axel; Helbich Thomas H; Kuriyama Nagato; Ostrowitzki S; Roberts Timothy P L; Shames David M; van Bruggen N; Wendland Michael F; Israel Mark A; Brasch Robert C. (Contrast Media Laboratory, Department of Radiology, University of California, San Francisco, California 94143-0628, USA.) Journal of magnetic resonance imaging : JMRI, (2002 Mar) 15 (3) 233-40. Journal code: 9105850. ISSN: 1053-1807. Pub. country: United States. Language: English.

AB PURPOSE: To evaluate the effects of a neutralizing anti-vascular endothelial growth factor (anti-**VEGF**) **antibody** on tumor microvascular permeability, a proposed indicator of angiogenesis, and tumor growth in a rodent malignant glioma model. MATERIALS AND

METHODS: A dynamic contrast-enhanced magnetic resonance **imaging** (MRI) technique, permitting noninvasive in vivo and in situ assessment of potential therapeutic effects, was used to measure tumor microvascular characteristics and volumes. U-87, a cell line derived from a human glioblastoma multiforme, was implanted orthotopically into brains of athymic homozygous nude rats. RESULTS: Treatment with the monoclonal antibody A4.6.1, specific for VEGF, significantly inhibited tumor microvascular permeability (6.1 +/- 3.6 mL min(-1)100 cc(-1)), compared to the control, saline-treated tumors (28.6 +/- 8.6 mL min(-1)100 cc(-1)), and significantly suppressed tumor growth (P <.05). CONCLUSION: Findings demonstrate that tumor vascular permeability and tumor growth can be inhibited by neutralization of endogenous VEGF and suggest that angiogenesis with the maintenance of endothelial hyperpermeability requires the presence of VEGF within the tissue microenvironment. Changes in tumor vessel permeability and tumor volumes as measured by contrast-enhanced MRI provide an assay that could prove useful for clinical monitoring of anti-angiogenic therapies in brain tumors.

L6 ANSWER 17 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:142492 Document No.: PREV200300142492. Safety and Efficacy of Intravitreal Injection of rhuFab VEGF in Combination With Verteporfin PDT on Experimental Choroidal Neovascularization. Gauthier, D. [Reprint Author]; Husain, D. [Reprint Author]; Kim, I. K. [Reprint Author]; Ezra, E. [Reprint Author]; Tsilimbaris, M. K. [Reprint Author]; Connolly, E. [Reprint Author]; Lane, A. M.; Gragoudas, E. S. [Reprint Author]; O'Neill, C. A.; Miller, J. W. [Reprint Author]. Retina Service, Angiogenesis Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA, USA. ARVO Annual Meeting Abstract Search and Program Planner, (2002) Vol. 2002, pp. Abstract No. 566. cd-rom.
Meeting Info.: Annual Meeting of the Association For Research in Vision and Ophthalmology. Fort Lauderdale, Florida, USA. May 05-10, 2002.
Language: English.

AB Purpose: To study the safety and efficacy of intravitreal injection of anti-**VEGF antibody** fragment (rhuFab VEGF) in combination with intravenous verteporfin photodynamic therapy (PDT) on experimental choroidal neovascularization in the monkey. Methods: Choroidal neovascularization was induced by laser injury in both eyes of cynomolgus monkeys and followed with weekly fundus photography and fluroescein angiography. Two weeks after induction, weekly treatments were started using intravitreal injection of rhuFab VEGF or placebo and PDT. Nine animals received intravitreal injections alternating with PDT. Six of these animals (group I) initially received intravitreal injections and were followed for 63 days. Three of these animals (group II) initially received PDT and were followed for 56 days. Two animals (group III) received injections and PDT the same day at two week intervals and were followed for 56 days. Fluorescein angiograms were graded using a masked, standardized protocol. The data were analyzed using the Stuart-Maxwell chi-square test for matched-pair analysis. Results: Three weeks after the start of treatment, 11 of 11 eyes treated with a combination of rhuFab VEGF injections and PDT showed no leakage from CNV on fluorescein angiography. This finding persisted for 6 weeks of follow-up. In those animals treated with placebo injections and PDT, 7 of 11 eyes showed no leakage from CNV, and 4 showed persistent leakage at 3 weeks. At 6 weeks, 9 of 11 eyes showed no leakage, and 2 eyes showed persistent leakage. Conclusion: Preliminary data indicate that intravitreal rhuFab VEGF in combination with verteporfin PDT causes greater reduction in angiographic leakage than PDT alone in experimental choroidal neovascularization.

L6 ANSWER 18 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:1681 Document No.: PREV200200001681. PET and PK analysis of the humanized monoclonal anti-**VEGF antibody** HuMV833. An EORTC-biological treatment development group phase I study. Jayson, Gordon

C. [Reprint author]; Zweit, Jamal; Mulatero, Clive; Hastings, David; Julyan, Peter; Ranson, Malcolm; Lawrance, Jeremy; McGown, Alan; Jackson, Alan; Hakannson, Leif; Groenwegen, Gerard; Lehmann, Frederick; Wagstaff, John; Levitt, Dan; Tang, Tao; Zwierzina, Heinz. Christie Hospital, Manchester, UK. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2001) Vol. 42, pp. 832-833. print.
Meeting Info.: 92nd Annual Meeting of the American Association for Cancer Research. New Orleans, LA, USA. March 24-28, 2001.
ISSN: 0197-016X. Language: English.

L6 ANSWER 19 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:495997 Document No.: PREV200100495997. Hepatocellular carcinoma: Correlation between vascular endothelial growth factor level and degree of enhancement by multiphase contrast-enhanced computed tomography. Kwak, Byung Kook; Shim, Hyung Jin; Park, Eon Sub; Kim, Soo Ah; Choi, Dongil; Lim, Hyo K.; Park, Cheol Keun; Chung, Jin Wook; Park, Jae Hyung [Reprint author]. Department of Radiology, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul, 110-744, South Korea. parkjh@radcom.snu.ac.kr. Investigative Radiology, (August, 2001) Vol. 36, No. 8, pp. 487-492. print.
CODEN: INVRAV. ISSN: 0020-9996. Language: English.

AB RATIONALE AND OBJECTIVES. To determine whether vascular endothelial growth factor (VEGF) is a histopathological factor influencing contrast enhancement of hepatocellular carcinoma (HCC) on computed tomography (CT). METHODS. Twenty-two nodular HCCs underwent multiphase helical CT and surgery. Tumor size, histological grading of differentiation, and type of hepatitis were evaluated. Tumor attenuation was graded as hyperattenuated, isoattenuated, and hypoattenuated. Immunohistochemical staining with anti-**VEGF antibody** was performed and scored as weak, intermediate, or strong. Spearman's rank correlation test was used. RESULTS. Tumors ranged from 1.0 to 12.0 cm (mean 5.1 cm). The degree of enhancement during the hepatic arterial phase was significantly correlated with VEGF expression. Size was negatively correlated with VEGF expression and the degree of enhancement, but histological grade and type of hepatitis were not correlated with VEGF expression, tumor size, or degree of enhancement. CONCLUSIONS. In HCC, VEGF expression is correlated with the degree of contrast enhancement during arterial-phase CT.

L6 ANSWER 20 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:221059 Document No.: PREV200100221059. Antibody neutralization of vascular endothelial growth factor inhibits wound granulation tissue formation. Howdieshell, Thomas R. [Reprint author]; Callaway, Dianne; Webb, Whitney L.; Gaines, Michael D.; Procter, Charles D., Jr.; Sathyanarayana; Pollock, Jennifer S.; Brock, Tommy L.; McNeil, Paul L.. Trauma/Surgical Critical Care, Medical College of Georgia, BA-4411, Augusta, GA, 30912, USA. thowdies@mail.mcg.edu. Journal of Surgical Research, (April, 2001) Vol. 96, No. 2, pp. 173-182. print.
CODEN: JSGRA2. ISSN: 0022-4804. Language: English.

AB Objective: The goal of this work was to test the functional role of vascular endothelial growth factor (VEGF) in promoting the vigorous granulation tissue formation, wound fluid accumulation, and angiogenic responses characteristic of this wound model. Background: Formation of vessel-rich granulation tissue is central to wound repair and is thought to be regulated by locally liberated angiogenic factors. Despite the clinical importance of granulation tissue formation in the early stage of wound healing, surprisingly little is known about the molecular identity of signals leading to granulation tissue invasion of a wound space. Methods: A ventral hernia, surgically created in the abdominal wall of 15 swine, was repaired using silicone sheeting and skin closure. An osmotic minipump, inserted in a remote subcutaneous pocket, delivered saline (n = 5), an irrelevant control antibody (n = 5), or neutralizing anti-**VEGF antibody** (n = 5) into the wound environment.

Serial ultrasonography on Days 2, 4, 7, 9, 11, and 14 was used to determine the dimensions of the subcutaneous granulation tissue and wound fluid compartment. VEGF and transforming growth factor beta1 (TGF-beta1) levels in serial wound fluid samples were quantitated by ELISA. On Day 14, animals were sacrificed and the abdominal wall was harvested for histologic, biochemical, and molecular analyses. Results: In animals receiving saline or an irrelevant antibody, a nearly linear 4-fold increase in granulation tissue thickness and 7-fold increase in wound fluid volume were measured over the 14-day study interval. In contrast, in animals receiving anti-VEGF neutralizing antibody, Day 14 granulation tissue thickness and wound fluid volume measurements were essentially unchanged from Day 2 values. Moreover, in the anti-VEGF animals, ultrasonography was unable to resolve the "angiogenic zone" typical of both controls, and correspondingly, wound vessel count and vascular surface area estimates derived from image analysis of histological sections were 3-fold lower in the anti-VEGF animals compared with the saline and antibody controls. Finally, VEGF levels in wound fluid detectable by ELISA analysis were strikingly (10-fold) reduced in anti-VEGF animals on Postsurgery Days 7-14. In contrast, TGF-beta1 levels were unaffected by the anti-VEGF treatment. Conclusion: Functional VEGF is a key mediator in wound angiogenesis, fluid accumulation, and granulation tissue formation.

L6 ANSWER 21 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:213383 Document No.: PREV200100213383. Correlation of VEGF with contrast enhancement on dual-phase dynamic helical CT in liver tumors: Preliminary study. Kwak, Byung Kook [Reprint author]; Shim, Hyung Jin; Park, Un Sub; Lee, Tae Jin; Paeng, Sung Suk; Lee, Chang Jun; Lim, Hyo K.; Park, Cheol Keun. Department of Radiology, Yongsan Hospital, Chung-Ang University College of Medicine, 65 Hangangro 3-ga, Yongsan-gu, Seoul, 140-757, South Korea. kbk6824@chollian.net. Journal of Korean Medical Science, (February, 2001) Vol. 16, No. 1, pp. 83-87. print.
CODEN: JKMSEH. ISSN: 1011-8934. Language: English.

AB The purpose of this preliminary study is to elucidate that vascular endothelial growth factor (VEGF) influences contrast enhancement of hepatic tumors on computed tomography (CT). Fourteen patients with hepatic tumors (11 hepatocellular carcinomas; 3 metastatic cancers) underwent a dual-phase dynamic helical CT or computed tomographic hepatic arteriography. The attenuation of each mass was determined as hyperattenuation, isoattenuation or hypoattenuation with respect to the adjacent nontumorous parenchyma. Gun-needle biopsy was done for each tumor, and paraffin sections were immunostained with anti- **VEGF antibody** by the avidin-biotin-peroxidase complex method. The pathologic grade was made by intensity (1+, 2+, 3+) and area (+-, 1+, 2+). The tumor ranged 2.0-14.0 cm in size (mean, 5.8 cm). In arterial phase, the intensity was not correlated with the degree of enhancement (p=0.086). However, the correlation between the attenuation value of hepatic arterial phase and the area of positive tumor cells was statistically significant (p=0.002). VEGF may be the factor that enhances the hepatic mass with water-soluble iodinated contrast agent in CT.

L6 ANSWER 22 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN

2000:772677 Document No. 133:349140 Compositions and methods for cancer treatment by selectively inhibiting VEGF. Thorpe, Philip E.; Brekken, Rolf A. (Board of Regents, the University of Texas System, USA). PCT Int. Appl. WO 2000064946 A2 20001102, 297 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US11367 20000428. PRIORITY: US 1999-PV131432

19990428.
AB Disclosed are antibodies that specifically inhibit VEGF binding to only one (VEGFR2) of the two VEGF receptors. The antibodies effectively inhibit angiogenesis and induce tumor regression, and yet have improved safety due to their specificity. The present invention thus provides new antibody-based compns., methods and combined protocols for treating cancer and other angiogenic diseases. Advantageous immunoconjugate and prodrug compns. and methods using the new VEGF-specific antibodies are also provided.

L6 ANSWER 23 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2001:79675 Document No.: PREV200100079675. Peritumoral brain edema associated with meningioma: influence of vascular endothelial growth factor expression and vascular blood supply. Yoshioka, H. [Reprint author]; Hama, S.; Yamasaki, F.; Taniguchi, E.; Sugiyama, K.; Arita, K.; Kurisu, K.. Hiroshima Univ Sch Med, Hiroshima, Japan. Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-189.6. print. Meeting Info.: 30th Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 04-09, 2000. Society for Neuroscience. ISSN: 0190-5295. Language: English.
AB BACKGROUND: The extent of peritumoral brain edema (PTBE) associated with meningiomas is very variable. Many causative factors have been investigated, but the mechanism of PTBE associated with meningioma has been unclear until now. Recently, the cerebral-pial blood supply and vascular endothelial growth factor (VEGF) have been implicated as causative factors of PTBE. METHODS: Seventy-three supratentorial meningiomas were investigated to identify factors, including type of arterial blood supply and VEGF expression, that may influence the development of meningioma-associated PTBE. The type of arterial blood supply was defined by the selective angiography. Paraffin embedded tumor sections were stained with monoclonal **VEGF antibody** by an immunoperoxidase method. The extent of PTBE was estimated by using preoperative magnetic resonance **imaging** as an edema index (EI). RESULTS: Forty-six meningiomas demonstrated PTBE, and the other 27 did not. Multiple regression analysis revealed close correlation between PTBE and type of arterial supply ($P = 0.004$), size of tumor ($P = 0.021$), vascular density ($P = 0.028$), and VEGF expression ($P = 0.046$). In meningiomas with cerebral-pial supply, the EI had increased significantly, just as VEGF was strongly expressed ($P < 0.001$). In contrast, meningiomas without a cerebral-pial supply developed little or no PTBE and less VEGF expression. CONCLUSIONS: The current results suggest that VEGF expression contributes to PTBE formation in meningioma only when a cerebral-pial blood supply exists.

L6 ANSWER 24 OF 28 MEDLINE on STN DUPLICATE 10
1999190194. PubMed ID: 10091773. Peritumoral brain edema associated with meningioma: influence of vascular endothelial growth factor expression and vascular blood supply. Yoshioka H; Hama S; Taniguchi E; Sugiyama K; Arita K; Kurisu K. (Department of Neurosurgery, Hiroshima University School of Medicine, Japan.) Cancer, (1999 Feb 15) 85 (4) 936-44. Journal code: 0374236. ISSN: 0008-543X. Pub. country: United States. Language: English.
AB BACKGROUND: The extent of peritumoral brain edema (PTBE) associated with meningiomas is very variable. Many causative factors have been investigated, but the mechanism of PTBE associated with meningioma has been unclear until now. Recently, the cerebral-pial blood supply and vascular endothelial growth factor (VEGF) have been implicated as causative factors of PTBE. METHODS: Seventy-three supratentorial meningiomas were investigated to identify factors, including type of arterial blood supply and VEGF expression, that may influence the development of meningioma-associated PTBE. The type of arterial blood supply was defined by the selective angiography. Paraffin embedded tumor sections were stained with monoclonal **VEGF antibody** by an immunoperoxidase method. The extent of PTBE was estimated by using preoperative magnetic resonance **imaging** as an edema index (EI).

RESULTS: Forty-six meningiomas demonstrated PTBE, and the other 27 did not. Multiple regression analysis revealed close correlation between PTBE and type of arterial supply ($P = 0.004$), size of tumor ($P = 0.021$), vascular density ($P = 0.028$), and VEGF expression ($P = 0.046$). In meningiomas with cerebral-pial supply, the EI had increased significantly, just as VEGF was strongly expressed ($P < 0.001$). In contrast, meningiomas without a cerebral-pial supply developed little or no PTBE and less VEGF expression. CONCLUSIONS: The current results suggest that VEGF expression contributes to PTBE formation in meningioma only when a cerebral-pial blood supply exists.

L6 ANSWER 25 OF 28 MEDLINE on STN DUPLICATE 11

1998289311. PubMed ID: 9626071. Mouse model of angiogenesis. Couffinhal T; Silver M; Zheng L P; Kearney M; Witzenbichler B; Isner J M. (Department of Medicine (Cardiology), St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston Massachusetts 02135, USA.) American journal of pathology, (1998 Jun) 152 (6) 1667-79. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB Neovascularization of ischemic muscle may be sufficient to preserve tissue integrity and/or function and may thus be considered to be therapeutic. The regulatory role of vascular endothelial growth factor (VEGF) in therapeutic angiogenesis was suggested by experiments in which exogenously administered VEGF was shown to augment collateral blood flow in animals and patients with experimentally induced hindlimb or myocardial ischemia. To address the possible contribution of postnatal endogenous VEGF expression to collateral vessel development in ischemia tissues, we developed a mouse model of hindlimb ischemia. The femoral artery of one hindlimb was ligated and excised. Laser Doppler perfusion **imaging** (LDPI) was employed to document the consequent reduction in hindlimb blood flow, which typically persisted for up to 7 days. Serial in vivo examinations by LDPI disclosed that hindlimb blood flow was progressively augmented over the course of 14 days, ultimately reaching a plateau between 21 and 28 days. Morphometric analysis of capillary density performed at the same time points selected for in vivo analysis of blood flow by LDPI confirmed that the histological sequence of neovascularization corresponded temporally to blood flow recovery detected in vivo. Endothelial cell proliferation was documented by immunostaining for bromodeoxyuridine injected 24 hours before each of these time points, providing additional evidence that angiogenesis constitutes the basis for improved collateral-dependent flow in this animal model. Neovascularization was shown to develop in association with augmented expression of VEGF mRNA and protein from skeletal myocytes as well as endothelial cells in the ischemic hindlimb; that such reparative angiogenesis is indeed dependent upon VEGF up-regulation was confirmed by impaired neovascularization after administration of a neutralizing **VEGF antibody.** Sequential characterization of the in vivo, histological, and molecular findings in this novel animal model thus document the role of VEGF as endogenous regulator of angiogenesis in the setting of tissue ischemia. Moreover, this murine model represents a potential means for studying the effects of gene targeting on nutrient angiogenesis in vivo.

L6 ANSWER 26 OF 28 MEDLINE on STN DUPLICATE 12

1998222974. PubMed ID: 9563510. Intercellular communication between vascular smooth muscle and endothelial cells mediated by heparin-binding epidermal growth factor-like growth factor and vascular endothelial growth factor. Abramovitch R; Neeman M; Reich R; Stein I; Keshet E; Abraham J; Solomon A; Marikovsky M. (Department of Biological Regulation, The Weizmann Institute of Science, Rehovot, Israel.) FEBS letters, (1998 Apr 3) 425 (3) 441-7. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Heparin-binding epidermal growth factor-like growth factor (HB-EGF), a potent mitogen and migration factor for vascular smooth muscle cells (SMC), promoted neovascularization in vivo in the rabbit cornea. MRI demonstrated quantitatively the angiogenic effect of HB-EGF when

introduced subcutaneously into nude mice. HB-EGF is not directly mitogenic to endothelial cells but it induced the migration of bovine endothelial cells and release of endothelial cell mitogenic activity from bovine vascular SMC. This mitogenic activity was specifically blocked by neutralizing anti-vascular endothelial growth factor (**VEGF**) **antibodies.** In contrast, EGF or transforming growth factor-alpha (TGF-alpha) had almost no effect on release of endothelial mitogenicity from SMC. In addition, RT-PCR analysis demonstrated that VEGF165 mRNA levels were increased in vascular SMC 4-10-fold by 0.35-2 nM of HB-EGF, respectively. Our data suggest that HB-EGF, as a mediator of intercellular communication, may play a new important role in supporting wound healing, tumor progression and atherosclerosis by stimulating angiogenesis.

L6 ANSWER 27 OF 28 MEDLINE on STN DUPLICATE 13
1998250918. PubMed ID: 9589031. Magnetic resonance **imaging** detects suppression of tumor vascular permeability after administration of antibody to vascular endothelial growth factor. Pham C D; Roberts T P; van Bruggen N; Melnyk O; Mann J; Ferrara N; Cohen R L; Brasch R C. (Department of Radiology, University of California, San Francisco, USA.) Cancer investigation, (1998) 16 (4) 225-30. Journal code: 8307154. ISSN: 0735-7907. Pub. country: United States. Language: English.

AB Macromolecular contrast medium-enhanced magnetic resonance **imaging** (MRI) and tumor-volume measurements were applied to monitor the effects of anti-vascular endothelial growth factor (anti-**VEGF**) **antibody** on microvascular characteristics and tumor growth of MDA-MB-435 human breast cancer cells implanted in nude rats. Administration of anti-**VEGF antibody** (three 1 mg doses at 3-day intervals) induced significant reductions in tumor growth rates ($p < 0.05$) and in MRI-assayed microvascular permeabilities ($p < 0.05$). Results of the study were consistent with previous observations that new microvessels formed in response to angiogenesis are hyperpermeable, and with the hypothesis that hyperpermeability is a mechanistic element in angiogenesis. Variations in tumor-vessel hyperpermeability can be measured by contrast-enhanced MRI, which may prove useful for assessing antiangiogenesis therapy.

L6 ANSWER 28 OF 28 MEDLINE on STN DUPLICATE 14
97191649. PubMed ID: 9039595. Assessing tumor angiogenesis using macromolecular MR **imaging** contrast media. Brasch R; Pham C; Shames D; Roberts T; van Dijke K; van Bruggen N; Mann J; Ostrowitzki S; Melnyk O. (Department of Radiology, University of California San Francisco 94143-0628, USA.) Journal of magnetic resonance imaging : JMRI, (1997 Jan-Feb) 7 (1) 68-74. Ref: 40. Journal code: 9105850. ISSN: 1053-1807. Pub. country: United States. Language: English.

AB MRI enhanced with a macromolecular contrast medium (MMCM) has previously been shown to estimate tumor microvascular characteristics that correlate closely with histologic microvascular density, an established surrogate of tumor angiogenesis. A similar MMCM-enhanced MRI technique has now been used to investigate the acute tumor microvascular effects of antibody-mediated inhibition of vascular endothelial growth factor (VEGF), a well-studied and potent angiogenesis stimulator. Athymic rats xenografted with a human breast carcinoma (MDA-MB-435) were imaged after administration of albumin-gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA30) using a heavily T1-weighted three dimensional-spoiled gradient-refocused acquisition in a steady-state pulse sequence before and 24 hours after treatment with anti-**VEGF antibody** (single dose of 1 mg). Changes in longitudinal relaxivity (delta R1) were analyzed using a bidirectional two-compartment kinetic model to estimate tumor fractional blood volume (fBV) and permeability surface area product (PS). Data showed a significant decrease ($P < 0.05$) of tumor PS with respect to macromolecular contrast medium at 24 hours after treatment with anti-**VEGF antibody.** No significant change was observed in fBV. Suppression of tumor microvascular permeability induced by anti-**VEGF antibody** can be detected and quantified

by MMCM-enhanced MRI. MRI grading of tumor angiogenesis and monitoring of anti-angiogenesis interventions could find wide clinical application.

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L7 7 L1 AND "VEGF-D"

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L8 6 DUP REMOVE L7 (1 DUPLICATE REMOVED)

=> d l8 1-6 cbib abs

L8 ANSWER 1 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
DUPLICATE 1
2003:292293 Document No.: PREV200300292293. **VEGF-D** is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. Rissanen, Tuomas T.; Markkanen, Johanna E.; Gruchala, Marcin; Heikura, Tommi; Puranen, Antti; Kettunen, Mikko I.; Kholova, Ivana; Kauppinen, Risto A.; Achen, Marc G.; Stacker, Steven A.; Alitalo, Kari; Yla-Herttuala, Seppo [Reprint Author]. Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute, University of Kuopio, FIN-70211, PO Box 1627, Kuopio, Finland. Seppo.Ylaherttuala@uku.fi. Circulation Research, (May 30 2003) Vol. 92, No. 10, pp. 1098-1106. print.
ISSN: 0009-7330 (ISSN print). Language: English.
AB Optimal angiogenic and lymphangiogenic gene therapy requires knowledge of the best growth factors for each purpose. We studied the therapeutic potential of human vascular endothelial growth factor (VEGF) family members VEGF-A, VEGF-B, VEGF-C, and **VEGF-D** as well as a VEGFR-3-specific mutant (VEGF-C156S) using adenoviral gene transfer in rabbit hindlimb skeletal muscle. The significance of proteolytic processing of **VEGF-D** was explored using adenoviruses encoding either full-length or mature (DELTANDELTAC) **VEGF-D**. Adenoviruses expressing potent VEGFR-2 ligands, VEGF-A and VEGF-DDELTANDELTAC, induced the strongest angiogenesis and vascular permeability effects as assessed by capillary vessel and perfusion measurements, modified Miles assay, and MRI. The most significant feature of angiogenesis induced by both VEGF-A and VEGF-DDELTANDELTAC was a remarkable enlargement of microvessels with efficient recruitment of pericytes suggesting formation of arterioles or venules. VEGF-A also moderately increased capillary density and created glomeruloid bodies, clusters of tortuous vessels, whereas VEGF-DDELTANDELTAC-induced angiogenesis was more diffuse. Vascular smooth muscle cell proliferation occurred in regions with increased plasma protein extravasation, indicating that arteriogenesis may be promoted by VEGF-A and VEGF-DDELTANDELTAC. Full-length VEGF-C and **VEGF-D** induced predominantly and the selective VEGFR-3 ligand VEGF-C156S exclusively lymphangiogenesis. Unlike angiogenesis, lymphangiogenesis was not dependent on nitric oxide. The VEGFR-1 ligand VEGF-B did not promote either angiogenesis or lymphangiogenesis. Finally, we found a positive correlation between capillary size and vascular permeability. This study compares, for the first time, angiogenesis and lymphangiogenesis induced by gene transfer of different human VEGFs, and shows that **VEGF-D** is the most potent member when delivered via an adenoviral vector into skeletal muscle.

L8 ANSWER 2 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2004:49067 Document No.: PREV200400050979. Comparison of the abundance of 10 radiation-induced proteins with their differential gene expression in L929 cells. Szkanderova, S.; Port, M. [Reprint Author]; Stulik, J.; Hernychova, L.; Kasalova, I.; Van Beuningen, D.; Abend, M.. Institute of Radiobiology, German Armed Forces, Neuherbergstr. 11, D-80937, Munich, Germany. matthias.port@web.de. International Journal of Radiation Biology, (August 2003) Vol. 79, No. 8, pp. 623-633. print.

ISSN: 0955-3002 (ISSN print). Language: English.

AB Purpose: To determine whether radiation-induced changes in protein abundance can be correlated with their differential gene expression in a murine fibroblast L929 cell line. Materials and methods: L929 cells were irradiated with 6 Gy. Cell lysates were collected at different points in time (20 min, 12, 24, 36, 48 and 72 h). The extracted proteins were separated by two-dimensional gel electrophoresis and quantified using computerized image analysis. Proteins exhibiting a differential expression equal to or more than twofold were identified by mass spectrometry following trypsin digestion. From these, 10 proteins characterized by large changes of radiation-induced abundance were selected in order to measure their corresponding gene expression using RTQ-PCR (real-time quantitative polymerase chain reaction). Results: Up to 15-fold changes in the abundance of these 10 proteins were associated with no detectable changes more than twofold on the gene expression level. However, one gene (**VEGF-D**) showed a significant (p=0.005) up-regulation (1.8-fold). Conclusions: Deducing protein abundance from mRNA expression levels and vice versa appears to be of limited use. Furthermore, examination of transcriptional and translational changes provides different but complementary information.

L8 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN

2002:555522 Document No. 137:119669 VEGFR-3 inhibitor materials and methods. Alitalo, Kari; Koivunen, Erkki; Kubo, Hajime (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2002057299 A2 20020725, 149 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-IB99 20020116. PRIORITY: US 2001-PV262476 20010117.

AB The present invention relates to the diagnostics, evaluation, and therapeutic intervention of disorders mediated by the activity of cell surface receptor VEGFR-3, which activity often is stimulated by VEGFR-3 ligands VEGF-C and **VEGF-D**. More particularly, the present invention identifies novel methods and compns. for the inhibition of VEGF-C/D binding to VEGFR-3. The compns. of the present invention will be useful the inhibition of angiogenesis and lymphangiogenesis. Many uses of such compds., for screening samples, **imaging**, diagnosis, and therapy, are also provided. For example, in one emobiment, the invention provides an isolated peptide comprising the formula: X1X2X3X4X5X6X7X8, wherein X1 through X8 are amino acid residues.

L8 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN

2001:545508 Document No. 135:132464 Cyclic peptide inhibitors of VEGF, VEGF-C, and **VEGF-D**, preparation methods, pharmaceutical compositions, and therapeutic use. Achen, Marc G.; Hughes, Richard A.; Stacker, Steven; Cendron, Angela (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2001052875 A1 20010726, 102 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US1533 20010118. PRIORITY: US 2000-PV176293 20000118; US 2000-PV204590 20000516.

AB The invention provides monomeric monocyclic peptide inhibitors and dimeric bicyclic peptide inhibitors based on exposed loop fragments of a growth factor protein, e.g. loop 1, loop 2 or loop 3 of **VEGF-D**, as well as methods of making them, pharmaceutical compns. containing them,

and therapeutic methods of use.

L8 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
2000:441581 Document No. 133:72945 Antibodies to truncated **VEGF-D** and uses thereof. Achen, Marc G.; Stacker, Steven Alan (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2000037025 A2 20000629, 44 pp. DESIGNATED STATES: W: AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US31332 19991221. PRIORITY: US 1998-PV113254 19981221; US 1999-PV134556 19990517.
AB The invention is based on the isolation of antibodies that were made to a polypeptide having the amino acid sequence for a truncated **VEGF-D**. One of these antibodies can interfere with the activity of **VEGF-D** mediated by VEGFR-2 and interfere with the binding of **VEGF-D** to VEGFR-3 but does not interfere with the activity of VEGF mediated by VEGFR-2 or bind to VEGF-C. The antibodies, antibody fragments or compns. containing the antibodies are useful for diagnosis, prognosis, and therapy of **VEGF-D** or VEGF-C related diseases, e.g. cancer, diabetic retinopathy, psoriasis, arthropathy, fluid accumulation in the heart and/or lung.

L8 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
2000:260057 Document No. 132:298824 Flt4 (VEGFR-3) as a target for tumor **imaging** and anti-tumor therapy. Alitalo, Kari; Kaipainen, Arja; Valltola, Reija; Jussila, Lotta (Ludwig Institute for Cancer Research, USA; Helsinki University Licensing Ltd. Oy). PCT Int. Appl. WO 2000021560 A1 20000420, 148 pp. DESIGNATED STATES: W: AU, CA, CN, JP, NO, NZ; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23525 19991008. PRIORITY: US 1998-169079 19981009.
AB The present invention provides purified Flt4 receptor tyrosine kinase polypeptides and fragments thereof, polynucleotides encoding such polypeptides, antibodies that specifically bind such polypeptides, and uses therefor.

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L11 ANSWER 1 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2002:334517 Document No.: PREV200200334517. Antibodies to truncated VEGF-D and thereof. **Achen, Marc G.** [Inventor, Reprint author]; **Stacker, Steven Alan** [Inventor]. Parkville, Australia. ASSIGNEE: Ludwig Institute for Cancer Research. Patent Info.: US 6383484 May 07, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (May 7, 2002) Vol. 1258, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention is based on the isolation of antibodies that were made to a polypeptide having the amino acid sequence for a truncated VEGF-D. One of these antibodies can interfere with the activity of VEGF-D mediated by VEGFR-2 and interfere with the binding of VEGF-D to VEGFR-3 but does not

interfere with the activity of VEGF mediated by VEGFR-2 or bind to VEGF-C. The invention provides pharmaceutical and diagnostic compositions and methods utilizing these antibodies.

L11 ANSWER 2 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2001:112744 Document No.: PREV200100112744. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. **Stacker, Steven A.** [Reprint author]; Caesar, Carol; Baldwin, Megan E.; Thornton, Gillian E.; Williams, Richard A.; Prevo, Remko; Jackson, David G.; Nishikawa, Shin-Ichi; Kubo, Hajime; **Achen, Marc G.**. Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Melbourne, VIC, Australia. steven.stacker@ludwig.edu.au. Nature Medicine, (February, 2001) Vol. 7, No. 2, pp. 186-191. print.
ISSN: 1078-8956. Language: English.
AB Metastasis to local lymph nodes via the lymphatic vessels is a common step in the spread of solid tumors. To investigate the molecular mechanisms underlying the spread of cancer by the lymphatics, we examined the ability of vascular endothelial growth factor (VEGF)-D, a ligand for the lymphatic growth factor receptor VEGFR-3/Flt-4, to induce formation of lymphatics in a mouse tumor model. Staining with markers specific for lymphatic endothelium demonstrated that VEGF-D induced the formation of lymphatics within tumors. Moreover, expression of VEGF-D in tumor cells led to spread of the tumor to lymph nodes, whereas expression of VEGF, an angiogenic growth factor which activates VEGFR-2 but not VEGFR-3, did not. VEGF-D also promoted tumor angiogenesis and growth. Lymphatic spread induced by VEGF-D could be blocked with an antibody specific for VEGF-D. This study demonstrates that lymphatics can be established in solid tumors and implicates VEGF family members in determining the route of metastatic spread.

L11 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 1
2000247148. PubMed ID: 10785369. Monoclonal antibodies to vascular endothelial growth factor-D block its interactions with both VEGF receptor-2 and VEGF receptor-3. **Achen M G**; Roufail S; Domagala T; Catimel B; Nice E C; Geleick D M; Murphy R; Scott A M; Caesar C; Makinen T; Alitalo K; **Stacker S A.** (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Victoria, Australia.. marc.achen@ludwig.edu.au) . European journal of biochemistry / FEBS, (2000 May) 267 (9) 2505-15. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB Vascular endothelial growth factor-D (VEGF-D), the most recently discovered mammalian member of the VEGF family, is an angiogenic protein that activates VEGF receptor-2 (VEGFR-2/Flk1/KDR) and VEGFR-3 (Flt4). These receptor tyrosine kinases, localized on vascular and lymphatic endothelial cells, signal for angiogenesis and lymphangiogenesis. VEGF-D consists of a central receptor-binding VEGF homology domain (VHD) and N-terminal and C-terminal propeptides that are cleaved from the VHD to generate a mature, bioactive form consisting of dimers of the VHD. Here we report characterization of mAbs raised to the VHD of human VEGF-D in order to generate VEGF-D antagonists. The mAbs bind the fully processed VHD with high affinity and also bind unprocessed VEGF-D. We demonstrate, using bioassays for the binding and cross-linking of VEGFR-2 and VEGFR-3 and biosensor analysis with immobilized receptors, that one of the mAbs, designated VD1, is able to compete potently with mature VEGF-D for binding to both VEGFR-2 and VEGFR-3 for binding to mature VEGF-D. This indicates that the binding epitopes on VEGF-D for these two receptors may be in close proximity. Furthermore, VD1 blocks the mitogenic response of human microvascular endothelial cells to VEGF-D. The **anti-(VEGF-D)** mAbs raised to the bioactive region of this growth factor will be powerful tools for analysis of the biological functions of VEGF-D.

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L15 ANSWER 1 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
DUPLICATE 1
2003:292293 Document No.: PREV200300292293. VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. Rissanen, Tuomas T.; Markkanen, Johanna E.; Gruchala, Marcin; Heikura, Tommi; Puranen, Antti; Kettunen, Mikko I.; Kholova, Ivana; Kauppinen, Risto A.; **Achen, Marc G.**; **Stacker, Steven A.**; Alitalo, Kari; Yla-Herttuala, Seppo [Reprint Author]. Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute, University of Kuopio, FIN-70211, PO Box 1627, Kuopio, Finland. Seppo.Ylaherttuala@uku.fi. Circulation Research, (May 30 2003) Vol. 92, No. 10, pp. 1098-1106. print.
ISSN: 0009-7330 (ISSN print). Language: English.
AB Optimal angiogenic and lymphangiogenic gene therapy requires knowledge of the best growth factors for each purpose. We studied the therapeutic potential of human vascular endothelial growth factor (VEGF) family members VEGF-A, VEGF-B, VEGF-C, and VEGF-D as well as a VEGFR-3-specific mutant (VEGF-C156S) using adenoviral gene transfer in rabbit hindlimb skeletal muscle. The significance of proteolytic processing of VEGF-D was explored using adenoviruses encoding either full-length or mature (DELTANDELTAC) VEGF-D. Adenoviruses expressing potent VEGFR-2 ligands, VEGF-A and VEGF-DDELTANDELTAC, induced the strongest angiogenesis and vascular permeability effects as assessed by capillary vessel and perfusion measurements, modified Miles assay, and MRI. The most significant feature of angiogenesis induced by both VEGF-A and VEGF-DDELTANDELTAC was a remarkable enlargement of microvessels with efficient recruitment of pericytes suggesting formation of arterioles or venules. VEGF-A also moderately increased capillary density and created glomeruloid bodies, clusters of tortuous vessels, whereas VEGF-DDELTANDELTAC-induced angiogenesis was more diffuse. Vascular smooth muscle cell proliferation occurred in regions with increased plasma protein extravasation, indicating that arteriogenesis may be promoted by VEGF-A and VEGF-DDELTANDELTAC. Full-length VEGF-C and VEGF-D induced predominantly and the selective VEGFR-3 ligand VEGF-C156S exclusively lymphangiogenesis. Unlike angiogenesis, lymphangiogenesis was not dependent on nitric oxide. The VEGFR-1 ligand VEGF-B did not promote either angiogenesis or lymphangiogenesis. Finally, we found a positive correlation between capillary size and vascular permeability. This study compares, for the first time, angiogenesis and lymphangiogenesis induced by gene transfer of different human VEGFs, and shows that VEGF-D is the most potent member when delivered via an adenoviral vector into skeletal muscle.

L15 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
2001:545508 Document No. 135:132464 Cyclic peptide inhibitors of VEGF, VEGF-C, and VEGF-D, preparation methods, pharmaceutical compositions, and therapeutic use. **Achen, Marc G.**; Hughes, Richard A.; **Stacker, Steven**; Cendron, Angela (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2001052875 A1 20010726, 102 pp.
DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,

LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US1533 20010118. PRIORITY: US 2000-PV176293 20000118; US 2000-PV204590 20000516.

AB The invention provides monomeric monocyclic peptide inhibitors and dimeric bicyclic peptide inhibitors based on exposed loop fragments of a growth factor protein, e.g. loop 1, loop 2 or loop 3 of VEGF-D, as well as methods of making them, pharmaceutical compns. containing them, and therapeutic methods of use.

L15 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN

2000:441581 Document No. 133:72945 Antibodies to truncated VEGF-D and uses thereof. **Achen, Marc G.; Stacker, Steven Alan** (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2000037025 A2 20000629, 44 pp. DESIGNATED STATES: W: AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US31332 19991221. PRIORITY: US 1998-PV113254 19981221; US 1999-PV134556 19990517.

AB The invention is based on the isolation of antibodies that were made to a polypeptide having the amino acid sequence for a truncated VEGF-D. One of these antibodies can interfere with the activity of VEGF-D mediated by VEGFR-2 and interfere with the binding of VEGF-D to VEGFR-3 but does not interfere with the activity of VEGF mediated by VEGFR-2 or bind to VEGF-C. The antibodies, antibody fragments or compns. containing the antibodies are useful for diagnosis, prognosis, and therapy of VEGF-D or VEGF-C related diseases, e.g. cancer, diabetic retinopathy, psoriasis, arthropathy, fluid accumulation in the heart and/or lung.

L15 ANSWER 4 OF 6 MEDLINE on STN

89194070. PubMed ID: 2930695. The detection of axillary lymph node metastases from breast cancer by radiolabelled monoclonal antibodies: a prospective study. Tjandra J J; Sacks N P; Thompson C H; Leyden M J; **Stacker S A**; Lichtenstein M; Russell I S; Collins J P; Andrews J T; Pietersz G A; +. (Department of Pathology, University of Melbourne, Parkville, Victoria, Australia.) British journal of cancer, (1989 Feb) 59 (2) 296-302. Journal code: 0370635. ISSN: 0007-0920. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In a prospective study to assess the accuracy of monoclonal immunoscintigraphy for the detection of axillary lymph node metastases in breast cancer, two murine monoclonal antibodies that react with human breast cancer (3E1.2 and RCC-1) were labelled with 131iodine, and the radiolabelled antibody was injected subcutaneously into the interdigital spaces of both hands of 40 patients, 36 of whom had breast cancer and the remaining four of whom had fibroadenoma (the normal, contralateral axilla was used as a control). Of the patients with breast cancer, the findings from the scintigraphy images were correlated with histopathology or cytology of the axillary lymph nodes; images were regarded as positive and hence indicative of lymph node metastases if the amount of background-subtracted radioactive count in axilla on the side of breast cancer exceeded the contralateral normal side by a ratio greater than or equal to 1.5:1.0 as assessed by computer analysis. Using this method, immunoscintigraphy had an overall sensitivity of 33% (23% with 131I-3E1.2 and 50% with 131I-RCC-1) for the detection of lymph node metastases and a specificity of 63% (67% with 131I-3E1.2 and 60% with 131I-RCC-1) with problems of non-specific uptake by presumably normal lymph nodes. The results of immunoscintigraphy obtained with 131I-RCC-1 (IgG) were superior to 131I-3E1.2 (IgM) although the accuracy of immunoscintigraphy using 131I-RCC-1 (56%) was not much better than preoperative clinical assessment (50%). However, there were cases when immunoscintigraphy using

radiolabelled antibody (IgM or IgG) detected axillary lymph node metastases not suspected by clinical examination. Thus it appears that while immunoscintigraphy may be a useful adjunct to preoperative clinical assessment and is simple and safe, a major improvement in its accuracy is needed before it can replace axillary dissection and histological examination in the accurate staging of axilla in breast cancer.

L15 ANSWER 5 OF 6 MEDLINE on STN
87051388. PubMed ID: 3916079. The diagnosis of human tumours with monoclonal antibodies. Teh J G; **Stacker S A**; Thompson C H; McKenzie I F. Cancer surveys, (1985) 4 (1) 149-84. Ref: 108. Journal code: 8218015. ISSN: 0261-2429. Pub. country: ENGLAND: United Kingdom. Language: English.

L15 ANSWER 6 OF 6 MEDLINE on STN
85059908. PubMed ID: 6150280. Immunoscintigraphy for detection of lymph node metastases from breast cancer. Thompson C H; Lichtenstein M; **Stacker S A**; Leyden M J; Salehi N; Andrews J T; McKenzie I F. Lancet, (1984 Dec 1) 2 (8414) 1245-7. Journal code: 2985213R. ISSN: 0140-6736. Pub. country: ENGLAND: United Kingdom. Language: English.
AB A radiolabelled monoclonal antibody that reacts with human breast cancer was injected into the web space of each hand in eight women with breast cancer, and the axillae were scanned 16-24 h later. Scans were positive in seven axillae with palpable lymph nodes and in two axillae with impalpable lymph nodes (metastases later confirmed by needle aspiration). The scan was negative in one axilla with a palpable mass, and here no tumour cells were obtained on needle aspiration. In a man with axillary lymphoma, no specific binding of antibody was observed. This technique could lead to earlier and more precise diagnosis of lymph node metastasis in patients with breast cancer.

=> s "VEGF-D" and antibody
L16 151 "VEGF-D" AND ANTIBODY

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L18 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
2003:282828 Document No. 138:298132 Modulators of VEGF or VEGFR binding to neuropilin-2, materials and methods for **detecting** said modulators, and therapeutic uses of the modulators.. Alitalo, Kari; Karkkainen, Marika; Karila, Kaisa (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). PCT Int. Appl. WO 2003029814 A2 20030410, 181 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-EP11069 20021001. PRIORITY: US 2001-PV326326 20011001.
AB The present invention relates to identifying modulators of VEGF-C binding to the nervous system transmembrane protein neuropilin-2 and materials and methods for **detecting** said modulators. A method of screening for modulators of binding between a neuropilin growth factor receptor and a VEGF-C polypeptide is claimed comprising steps of: (a) contacting a neuropilin composition with a VEGF-C composition, in the presence and in the absence

of a putative modulator compound; (b) **detecting** binding between the neuropilin polypeptide and the VEGF-C polypeptide in the presence and absence of the putative modulator compound; and (c) identifying a modulator compound based on a decrease or increase in binding in the presence of the putative modulator compound as compared to binding in the absence of the putative modulator compound The neuropilin receptor composition comprises a neuropilin receptor extracellular domain fragment bound to a solid support or a neuropilin receptor extracellular domain fragment fused to an Ig Fc fragment. The VEGF-C composition comprises a purified mammalian prepro-VEGF-C polypeptide or a fragment. A method of screening for modulators of binding between a neuropilin growth factor receptor and a VEGFR-3 polypeptide is also claimed. The VEGFR-3 composition used in the method comprises a receptor extracellular domain fragment bound to a solid support or a receptor extracellular domain fragment fused to an Ig Fc fragment. Addnl. claimed is a method for screening for selectivity of a modulator of VEGF-C, VEGFR, or neuropilin biol. activity. A method of modulating growth, migration, or proliferation of cells, specifically neurons, in a mammalian organism by administering a composition comprising a neuropilin polypeptide or fragment, and a VEGF, a PlGF, a semaphorin, or a bispecific **antibody** specific for the neuropilin receptor and for a VEGF-C polypeptide or for a neuropilin receptor and a VEGFR is also claimed.

L18 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
2002:575554 Document No. 137:135068 Methods for treating neoplastic disease characterized by vascular endothelial growth factor D expression, for screening for neoplastic disease or metastatic risk, and for maintaining vascularization of tissue. Achen, Marc; Stacker, Steven (Australia). U.S. Pat. Appl. Publ. US 2002102260 A1 20020801, 45 pp., Cont.-in-part of U.S. Ser. No. 796,714. (English). CODEN: USXXCO. APPLICATION: US 2001-956095 20010920. PRIORITY: US 2000-PV186361 20000302; US 2000-PV234196 20000920; US 2001-796714 20010302.
AB A method for treating and alleviating disease characterized by the expression of **VEGF-D** involving screening to find an organism with tumor cells expressing **VEGF-D** and administering an effective amount of a **VEGF-D** antagonist; a method for screening for neoplastic disease, where detection of **VEGF-D** on or in a sample such as tumor cells, blood vessel endothelial cells or lymph vessel endothelial cells indicates neoplastic disease; a method for promoting and maintaining vascularization of normal tissue in an organism involving administering a vascularization promoting amount of **VEGF-D** or a fragment or analog thereof to the organism; a method for screening tumors for metastatic risk involving **detecting** expression of VEFG-D by a tumor which indicates metastatic risk; and a method of **detecting** micro-metastasis of neoplastic disease involving detection of **VEGF -D** on or in a tissue sample which indicates metastasis of a neoplastic disease.

L18 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
2001:661270 Document No. 135:205534 Methods for treating, screening for, and **detecting** cancers expressing vascular endothelial growth factor D (**VEGF-D**). Achen, Marc; Stacker, Steven (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2001064235 A1 20010907, 78 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US6791 20010302. PRIORITY: US 2000-PV186361 20000302.
AB A method for treating and alleviating melanomas and various cancers characterized by the expression of **VEGF-D** by the tumor

comprises screening to find an organism with tumor cells expressing **VEGF-D** and administering an effective amount of a **VEGF-D** antagonist to prevent binding of **VEGF-D**. Also provided are methods for screening for neoplastic diseases, where detection of **VEGF-D** on or in cells such as tumor cells, blood vessel endothelial cells, lymph vessel endothelial cells, and/or cells with potential neoplastic growth indicates neoplastic disease; a method for promoting and maintaining vascularization of normal tissue in an organism by administering **VEGF-D** or a fragment or analog thereof; methods for screening tumors for metastatic risk where expression of **VEGF-D** by the tumor indicates metastatic risk; and methods to detect micro-metastasis of neoplastic disease where detection of **VEGF-D** on or in a tissue sample indicates metastasis of neoplastic disease.

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=> s "VEGF receptor-3"
L19         204 "VEGF RECEPTOR-3"

=> s l19 and anti-VEGFR-3
L20           3 L19 AND ANTI-VEGFR-3

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PROCESSING COMPLETED FOR L20
L21           3 DUP REMOVE L20 (0 DUPLICATES REMOVED)

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L21  ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
2004:591422  Document No. 141:204543  Immunodetection and quantification of
     vascular endothelial growth factor receptor-3 in human malignant tumor
     tissues.  Bando, Hiroko; Brokelmann, Maren; Toi, Masakazu; Alitalo, Kari;
     Sleeman, Jonathan P.; Sipos, Bence; Groene, Hermann-Josef; Weich, Herbert
     A. (Department of Gene Regulation and Differentiation, National Research
     Centre for Biotechnology (GBF), Braunschweig, Germany).  International
     Journal of Cancer, 111(2), 184-191 (English) 2004.  CODEN: IJCNAW.  ISSN:
     0020-7136.  Publisher: Wiley-Liss, Inc..
AB   Vascular endothelial growth factor receptor-3 (VEGFR-3) and its ligands,
     vascular endothelial growth factor-C (VEGF-C) and -D (VEGF-D), are the
     major mols. involved in developmental and pathol. lymphangiogenesis.  Here
     the authors describe for the first time the development of a specific
     indirect ELISA for the quantification of VEGFR-3 in different human cell
     and tissue lysates.  A combination of the goat polyclonal anti-
     VEGFR-3 antibody and the mouse monoclonal anti-human
     VEGFR-3 antibody was used.  The assay was highly sensitive and
     reproducible with a detection range of 0.2-25 ng/mL.  The assay was
     specific for VEGFR-3, with no cross-reactivity to VEGFR-1 or VEGFR-2.
     Complex formation with VEGF-C and VEGF-D had no effect on the sensitivity
     of the assay.  The VEGFR-3 concentration in the lysates of cultured human
dermal
     microvascular endothelial cells was 14-fold higher than in the lysates
     from human umbilical vein endothelial cells.  In human kidney, breast,
     colon, gastric and lung cancer tissues the protein levels of VEGFR-3 were
     in the range of 0.6-16.7 ng/mg protein.  Importantly, the level of VEGFR-3
     protein detected in the ELISA correlated significantly with the number of
     VEGFR-3 pos. vessels observed in histochem. sections, suggesting that the
     ELISA assay may be a reliable surrogate of measuring VEGFR-3-pos. vessel
     d.  The protein levels of VEGFR-3 in 27 renal cell carcinoma samples had a
     significant correlation with the levels of VEGF-C, or biol. active, free
     VEGF-A, but not with VEGFR-I or total VEGF-A.  This assay provides a
     useful tool for the investigations of the expression levels of VEGFR-3 in
     physiol. and pathol. processes, particular in cancer and in
     lymphangiogenesis-related disease.

L21  ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
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2002:519260 Document No. 137:211309 Blockade of vascular endothelial growth factor receptor-3 signaling inhibits fibroblast growth factor-2-induced lymphangiogenesis in mouse cornea. Kubo, Hajime; Cao, Renhai; Brakenhielm, Ebba; Makinen, Taija; Cao, Yihai; Alitalo, Kari (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute and Helsinki University Central Hospital, Biomedicum Helsinki, University of Helsinki, Helsinki, 00014, Finland). Proceedings of the National Academy of Sciences of the United States of America, 99(13), 8868-8873 (English) 2002. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB Vascular endothelial growth factor receptor-3 (VEGFR-3) is a major mediator of lymphangiogenesis. Recently, VEGFR-3 ligands, VEGF-C, and VEGF-D were reported to promote tumor lymphangiogenesis and lymphatic metastasis, and these processes were inhibited by blocking of the VEGFR-3-signaling pathway. Here, we have adapted the mouse corneal angiogenesis assay to study potential lymphangiogenic factors and inhibitors. Immunohistochem. anal. with lymphatic endothelial markers showed that VEGF-C induces lymphatic as well as blood vessel growth in the cornea. By contrast, VEGF induced angiogenesis but not lymphangiogenesis. Fibroblast growth factor-2 (FGF-2) stimulated both lymphangiogenesis and angiogenesis. FGF-2 up-regulated VEGF-C expression in vascular endothelial and perivascular cells. Furthermore, administration of blocking **anti-VEGFR-3** antibodies inhibited the FGF-2-induced lymphangiogenesis. These findings show that VEGFR-3 can mediate lymphangiogenesis induced by other growth factors. Because increased expression of FGF-2 and VEGF-C has been associated with lymphatic metastasis, our results provide a potential strategy for the inhibition of lymphatic metastasis in cancer therapy.

L21 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN

2003:764019 Document No. 140:229829 Expression of vascular endothelial growth factor receptor-3 in the conjunctiva-a potential link between lymph-angiogenesis and leukocyte-trafficking on the ocular surface. Hamrah, Pedram; Zhang, Qiang; Dana, M. Reza (Laboratory of Immunology Schepens Eye Research Institute and the Department of Ophthalmology, Harvard Medical School, Boston, MA, USA). Advances in Experimental Medicine and Biology, 506(Lacrimal Gland, Tear Film, and Dry Eye Syndromes 3, Part B), 851-860 (English) 2002. CODEN: AEMBAP. ISSN: 0065-2598. Publisher: Kluwer Academic/Plenum Publishers.

AB The expression of vascular endothelial growth factor receptor-3 (VEGFR-3) in the conjunctiva was examined including its possible role in lymph-angiogenesis, using highly sensitive confocal microscopy techniques. VEGFR-3 was not only expressed by the lymphatic vasculature but was also expressed on almost all monocytic-derived cells in the conjunctiva. A subpopulation of the vessels pos. for the pan-endothelial marker CD31 was identified by **anti-VEGFR-3** labeling. Most of the VEGFR-3 expression did not relate to lymphatic endothelial expression, instead, it relates to the expression on monocytic-derived cells. In the normal uninflamed eye, VEGFR-3 could play a role in the trafficking of lymphatic endothelial cells in the ocular surface when these cells secrete VEGF-C. In the inflamed eye, VEGF-C induced the migration of the monocytic-derived cells into the cornea when it was addnl. secreted by inflammatory cells. VEGFR-3 could function as a "trap" or functional "decoy" receptor by binding VEGF-C and VEGF-D growth factors to prevent lymph-angiogenesis and angiogenesis into the clear cornea.

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L22 47 L19 AND ANTIBODY

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L23 20 DUP REMOVE L22 (27 DUPLICATES REMOVED)

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L23 ANSWER 1 OF 20 MEDLINE on STN DUPLICATE 1
2004270721. PubMed ID: 15150322. Involvement of the **VEGF receptor 3** in tubular morphogenesis demonstrated with a human anti-human VEGFR-3 monoclonal **antibody** that antagonizes receptor activation by VEGF-C. Persaud Kris; Tille Jean-Christophe; Liu Meilin; Zhu Zhenping; Jimenez Xenia; Pereira Daniel S; Miao Hua-Quan; Brennan Laura A; Witte Larry; Pepper Michael S; Pytowski Bronislaw. (ImClone Systems Incorporated, New York, NY 10014, USA.) Journal of cell science, (2004 Jun 1) 117 (Pt 13) 2745-56. Journal code: 0052457. ISSN: 0021-9533. Pub. country: England: United Kingdom. Language: English.

AB In this report we utilize a novel antagonist **antibody** to the human VEGFR-3 to elucidate the role of this receptor in in vitro tubular morphogenesis of bovine and human endothelial cells (EC cells) induced by VEGF-C. The **antibody** hF4-3C5 was obtained by panning a human phage display library on soluble human VEGFR-3. The binding affinity constant of hF4-3C5 significantly exceeds that of the interaction of VEGFR-3 with VEGF-C. hF4-3C5 strongly inhibits the binding of soluble VEGFR-3 to immobilized VEGF-C and abolishes the VEGF-C-mediated mitogenic response of cells that expresses a chimeric human VEGFR-3-cFMS receptor. In fluorescence experiments, hF4-3C5 reactivity is observed with human lymphatic endothelial cells (LECs) and human umbilical vein endothelial cells (HUVECs). Binding of hF4-3C5 shows that about half of bovine aortic endothelial (BAE) cells express VEGFR-3 and cells in this subpopulation are primarily responsible for the chemotactic response to the mature form of VEGF-C (VEGF-C(DeltaNDeltaC)). This response was strongly inhibited by the addition of hF4-3C5. In vitro tube formation by BAE cells induced by VEGF-C(DeltaNDeltaC) was reduced by greater than 60% by hF4-3C5 whereas the response to VEGF(165) was unaffected. Addition of hF4-3C5 together with an antagonist **antibody** to VEGFR-2 completely abolished the response to VEGF-C(DeltaNDeltaC). Similar results were obtained with HUVECs. Together, these findings point to a role for VEGFR-3 in vascular tubular morphogenesis and highlight the utility of hF4-3C5 as a tool for the investigation of the biology of VEGFR-3.

L23 ANSWER 2 OF 20 MEDLINE on STN DUPLICATE 2
2004496111. PubMed ID: 15466359. Vascular endothelial growth factor receptor-3 in hypoxia-induced vascular development. Nilsson Ingrid; Rolny Charlotte; Wu Yan; Pytowski Bronislaw; Hicklin Dan; Alitalo Kari; Claesson-Welsh Lena; Wennstrom Stefan. (Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden.) FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (2004 Oct) 18 (13) 1507-15. Journal code: 8804484. ISSN: 1530-6860. Pub. country: United States. Language: English.

AB Reduced tissue oxygen tension (hypoxia) is appreciated as an efficient stimulus for neovascularization. The effect of hypoxia on the very first stages of vascular development is, however, less well characterized. Here we show that hypoxic conditions (1% O2) potently stimulated formation of an extensive vascular network during a discrete stage of mouse embryonal stem cell differentiation. The morphological changes correlated with an expanding pool of endothelial cells and with activation of the vascular endothelial growth factor-d (Vegf-d) and **Vegf receptor -3** genes. **VEGF receptor-3** expression was confined to vascular endothelial cells and analysis of the lymphatic marker Prox-1 revealed no expansion of lymphatic endothelial cells. Administration of neutralizing **antibodies** against either **VEGF receptor-3** or VEGF receptor-2 impaired vascular network formation, whereas neutralizing **antibodies** against VEGF receptor-1 potentiated development of immature vascular structures. In addition, sequestering of **VEGF receptor -3** ligands reduced vascularization in a manner similar to neutralization of **VEGF receptor-3.** We conclude that hypoxia-driven vascular development requires the activity of **VEGF receptor-3.**

L23 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2005 ACS on STN
2004:591422 Document No. 141:204543 Immunodetection and quantification of vascular endothelial growth factor receptor-3 in human malignant tumor tissues. Bando, Hiroko; Brokelmann, Maren; Toi, Masakazu; Alitalo, Kari; Sleeman, Jonathan P.; Sipos, Bence; Groene, Hermann-Josef; Weich, Herbert A. (Department of Gene Regulation and Differentiation, National Research Centre for Biotechnology (GBF), Braunschweig, Germany). International Journal of Cancer, 111(2), 184-191 (English) 2004. CODEN: IJCNAW. ISSN: 0020-7136. Publisher: Wiley-Liss, Inc..

AB Vascular endothelial growth factor receptor-3 (VEGFR-3) and its ligands, vascular endothelial growth factor-C (VEGF-C) and -D (VEGF-D), are the major mols. involved in developmental and pathol. lymphangiogenesis. Here the authors describe for the first time the development of a specific indirect ELISA for the quantification of VEGFR-3 in different human cell and tissue lysates. A combination of the goat polyclonal anti-VEGFR-3 **antibody** and the mouse monoclonal anti-human VEGFR-3 **antibody** was used. The assay was highly sensitive and reproducible with a detection range of 0.2-25 ng/mL. The assay was specific for VEGFR-3, with no cross-reactivity to VEGFR-1 or VEGFR-2. Complex formation with VEGF-C and VEGF-D had no effect on the sensitivity of the assay. The VEGFR-3 concentration in the lysates of cultured human dermal microvascular endothelial cells was 14-fold higher than in the lysates from human umbilical vein endothelial cells. In human kidney, breast, colon, gastric and lung cancer tissues the protein levels of VEGFR-3 were in the range of 0.6-16.7 ng/mg protein. Importantly, the level of VEGFR-3 protein detected in the ELISA correlated significantly with the number of VEGFR-3 pos. vessels observed in histochem. sections, suggesting that the ELISA assay may be a reliable surrogate of measuring VEGFR-3-pos. vessel d. The protein levels of VEGFR-3 in 27 renal cell carcinoma samples had a significant correlation with the levels of VEGF-C, or biol. active, free VEGF-A, but not with VEGFR-I or total VEGF-A. This assay provides a useful tool for the investigations of the expression levels of VEGFR-3 in physiol. and pathol. processes, particular in cancer and in lymphangiogenesis-related disease.

L23 ANSWER 4 OF 20 MEDLINE on STN DUPLICATE 3
2003447242. PubMed ID: 14507895. Influence of photodynamic therapy on expression of vascular endothelial growth factor (VEGF), **VEGF receptor 3**, and pigment epithelium-derived factor.
Schmidt-Erfurth Ursula; Schlotzer-Schrehard Ursula; Cursiefen Claus; Michels Stephan; Beckendorf Arne; Naumann Gottfried O H. (University Eye Hospital Lubeck, Lubeck, Germany.. uschmidterfurth@ophtha.mu-luebeck.de) Investigative ophthalmology & visual science, (2003 Oct) 44 (10) 4473-80. Journal code: 7703701. ISSN: 0146-0404. Pub. country: United States. Language: English.

AB PURPOSE. To evaluate the impact of photodynamic therapy (PDT) on expression and distribution of vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR)-3, and pigment epithelium-derived factor (PEDF). METHODS. Eyes of patients scheduled for enucleation due to untreatable malignancy served as study eyes (n = 4), age-matched donor eyes were used as the control (n = 4). PDT using verteporfin with the recommended standard parameters was applied to intact areas of the perimacular region. Lesions were classified by ophthalmoscopy, fluorescein angiography (FA), and indocyanine green angiography (ICGA), as well as light and electron microscopic (LM/EM) histology. Immunolabeling using specific **antibodies** against VEGF, VEGFR-3, and PEDF was performed in PDT-treated areas, untreated collateral areas in study eyes, and untreated areas of control eyes. Specimens were fixed in 4% paraformaldehyde and 1% glutaraldehyde and embedded in paraffin. Four-micrometer-thick sections were stained using the peroxidase-labeled streptavidin-biotin method. RESULTS. All PDT-treated areas demonstrated characteristic choroidal hypofluorescence by FA and ICGA. LM/EM histology revealed selective damage of choriocapillary endothelial cells. VEGF was expressed in the

endothelial layer of choriocapillaries and focally within larger choroidal vessels in treated areas, but not in untreated areas. Sites with positive VEGF labeling also demonstrated upregulation of VEGFR-3. PEDF expression was localized to retinas in all eyes; however, PEDF staining of choroidal endothelial cells was specific for treated areas of study eyes. CONCLUSIONS. PDT using verteporfin induces a reproducible angiogenic response in elderly human eyes. VEGF, VEGFR-3, and PEDF expression is enhanced after PDT. Choroidal endothelial cells appear to be the primary site of angiogenic stimulation.

L23 ANSWER 5 OF 20 MEDLINE on STN DUPLICATE 4

2003081301. PubMed ID: 12393458. Modulation of VEGFR-2-mediated endothelial-cell activity by VEGF-C/VEGFR-3. Matsumura Kazuyoshi; Hirashima Masanori; Ogawa Minetaro; Kubo Hajime; Hisatsune Hiroshi; Kondo Nobuyuki; Nishikawa Satomi; Chiba Tsutomu; Nishikawa Shin-Ichi. (Division of Gastroenterology and Hepatology, Department of Internal Medicine, Graduate School of Medicine, Kyoto University, Japan.. kazuy@kuhp.hyoto-u.ac.jp) . Blood, (2003 Feb 15) 101 (4) 1367-74. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (**VEGF**) **receptor** 3 (VEGFR-3), a receptor for VEGF-C, was shown to be essential for angiogenesis as well as for lymphangiogenesis. Targeted disruption of the VEGFR-3 gene in mice and our previous study using an antagonistic monoclonal **antibody** (MoAb) for VEGFR-3 suggested that VEGF-C/VEGFR-3 signals might be involved in the maintenance of vascular integrity. In this study we used an in vitro embryonic stem (ES) cell culture system to maintain the VEGFR-3(+) endothelial cell (EC) and investigated the role of VEGFR-3 signals at the cellular level. In this system packed clusters of ECs were formed. Whereas addition of exogenous VEGF-A induced EC dispersion, VEGF-C, which can also stimulate VEGFR-2, promoted EC growth without disturbing the EC clusters. Moreover, addition of AFL4, an antagonistic MoAb for VEGFR-3, resulted in EC dispersion. Cytological analysis showed that VEGF-A- and AFL4-treated ECs were indistinguishable in many aspects but were distinct from the cytological profile induced by antagonistic MoAb for VE-cadherin (VECD-1). As AFL4-induced EC dispersion requires VEGF-A stimulation, it is likely that VEGFR-3 signals negatively modulate VEGFR-2. This result provides new insights into the involvement of VEGFR-3 signals in the maintenance of vascular integrity through modulation of VEGFR-2 signals. Moreover, our findings suggest that the mechanisms underlying AFL4-induced EC dispersion are distinct from those underlying VECD-1-induced dispersion for maintenance of EC integrity.

L23 ANSWER 6 OF 20 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:134135 Document No.: PREV200400132208. Cross-talk between the cAMP and calcium dependent signaling pathways regulates VEGF-A-stimulated normal rat cholangiocyte proliferation. Glaser, Shannon [Reprint Author]; Francis, Heather [Reprint Author]; Phinizy, Jo Lynne [Reprint Author]; Taffetani, Silvia [Reprint Author]; Venter, Julie; Baumann, Brandy; Alvaro, Domenico; Marzioni, Marco; Fava, Giammarco; Reichenbach, Ramona; Alpini, Gianfranco. Scott and White Hospital, Temple, TX, USA. Hepatology, (October 2003) Vol. 38, No. 4 Suppl. 1, pp. 686A. print. Meeting Info.: 54th Annual Meeting of the American Association for the Study of Liver Diseases. Boston, MA, USA. October 24-28, 2003. American Association for the Study of Liver Diseases. ISSN: 0270-9139 (ISSN print). Language: English.

AB Cholangiocytes are the target cells in cholangiopathies. The progression of cholangiopathies begins with initial proliferation of cholangiocytes followed by disappearance of cholangiocytes resulting in ductopenia. Cross-talk between Ca2+/PKC and adenylyl cyclase (which modulates the changes in cAMP levels) regulates cholangiocyte proliferation through changes in the PKA/Src/MEK/ERK1/2 pathway. We have shown that: (i) cholangiocytes from bile duct ligated (BDL) rats express and secrete

VEGF-A; (ii) cholangiocytes express VEGF receptors (i.e., VEGFR-2 and VEGFR-3); and (iii) chronic administration of an anti-VEGF-A **antibody** to BDL rats blocks cholangiocyte proliferation. However, our previous studies did not demonstrate: (i) if VEGF-A stimulates proliferation of normal cholangiocytes by directly interacting with VEGF receptors; and (ii) the signaling mechanisms for the VEGF-A induced proliferative response in normal rat cholangiocytes. We posed these questions: (i) Does chronic administration of recombinant VEGF-A to normal rats increase cholangiocyte proliferation and cAMP levels (a determinant of cholangiocyte proliferation)? and (ii) Does VEGF-A stimulate in vitro the proliferation of normal rat cholangiocytes through cross-talk between the IP3/Ca2+-dependent PKC and the cAMP-dependent ERK1/2 pathway? Methods: In in vivo studies, normal rats were treated by daily IP injections with recombinant VEGF-A (12 mug/rat) or saline for 1 week. Subsequently, we evaluated cholangiocyte proliferation by measurement of: (i) the number of CK-19 positive cholangiocytes and gamma-GT-positive ducts in liver sections; and (ii) PCNA protein expression in cholangiocytes. For the in vitro experiments, we used a polarized cultured system of normal rat intrahepatic cholangiocytes (NRIC), which we have developed (Am J Physiol 284:G1066-G1073, 2003). The time dependent stimulation of NRIC proliferation by VEGF-A was determined by stimulating NRIC with VEGF-A (100 nM) for 12, 24, 48 and 72 hours. NRIC proliferation was measured by PCNA protein expression by immunoblotting and MTS proliferation assay. VEGF-A-modulated proliferation of NRIC was also measured in the presence and absence of BAPTA/AM (5 muM, an intracellular Ca2+ chelator) or Go6976 (1 muM, a PKC inhibitor) for 48 hours. Proliferation was measured by PCNA protein expression and MTS proliferation assay. We measured the effect of VEGF-A on intracellular IP3 and Ca2+ levels. Intracellular cAMP levels (by RIA), and total and phosphorylated PKA and ERK1/2 protein expression (by immunoblots) were determined in NRIC stimulated with VEGF-A (100 nM) for 48 hours in the presence and absence of BAPTA/AM and Go6976. In NRIC treated with VEGF-A for 48 hours, we measured protein expression for the Ca2+-dependent PKC-alpha, which regulates cholangiocyte proliferation. Translocation of Ca2+-dependent PKC-alpha was determined by immunoblots in a triton-soluble and a triton-insoluble fraction from NRIC stimulated with VEGF. Results: Chronic administration of VEGF-A to normal rats increased cholangiocyte proliferation and cAMP levels compared to control rats. In vitro, VEGF-A increased IP3 and Ca2+ levels of NRIC. VEGF-A stimulated NRIC proliferation in a time course dependent fashion. Consistent with the concept that cross-talk between Ca2+/PKC and adenylyl cyclase regulates cholangiocyte proliferation through changes in the cAMP-dependent PKA/Src/MEK/ERK1/2 pathway, VEGF-A stimulated cholangiocyte proliferation and cAMP levels, and phosphorylation of PKA and ERK1/2 were all blocked by BAPTA/AM and Go6976. Furthermore, VEGF-A effects on cholangiocyte functions were associated with translocation of PKC-alpha. Conclusion: Modulation of the crosstalk between intracellular Ca2+ and adenylyl cyclase by VEGF-A may be an important approach for modulating cholangiocyte proliferation in cholangiopathies.

L23 ANSWER 7 OF 20 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:814471 The Genuine Article (R) Number: 722UA. Potent inhibition of angiogenesis by D,L-peptides derived from vascular endothelial growth factor receptor 2. Piossek C; Thierauch K H; Schneider-Mergener J; Volkmer-Engert R; Bachmann M F; Korff T; Augustin H G; Germeroth L (Reprint). IBA GmbH, Rudolf Wissell Str 28, D-37079 Gottingen, Germany (Reprint); IBA GmbH, D-37079 Gottingen, Germany; Cytos Biotechnol AG, Zurich, Switzerland; Schering AG, Res Labs, Berlin, Germany; Humboldt Univ, Univ Klinikum Charite, Inst Med Immunol, Berlin, Germany; Jerini AG, Berlin, Germany; Tumor Biol Ctr, Inst Mol Oncol, Dept Vasc Biol & Angiogenesis Res, Freiburg, Germany. THROMBOSIS AND HAEMOSTASIS (SEP 2003) Vol. 90, No. 3, pp. 501-510. Publisher: SCHATTAUER GMBH-VERLAG MEDIZIN NATURWISSENSCHAFTEN. HOLDERLINSTRASSE 3, D-70174 STUTTGART, GERMANY. ISSN: 0340-6245. Pub. country: Germany; Switzerland. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Vascular endothelial growth factor (VEGF) is a potent mitogen for endothelial cells and plays a central role in angiogenesis and vasculogenesis. Therefore,VEGF and its receptors VEGFR-1 and VEGFR-2 are prime targets for anti-angiogenic intervention which is thought to be one of the most promising approaches in cancer therapy. Recently, we have discovered a VEGFR-2-derived peptide ((247)RTELNVGlDFNWEYP(261)) representing a potential binding site to VEGF Using the spot synthesis technique, systematic D-amino acid substitutional analyses of this peptide were conducted and the resulting D,L-peptides inhibit VEGF binding to VEGFR-2 at half maximal concentration of 30 nM. The serum-stable D,L-peptides further inhibited autophosphorylation of the VEGFR-2 at nanomolar concentrations. Testing of the peptides in a spheroid-based angiogenesis assay demonstrated a potent anti-angiogenic effect in vitro. The rational design of potent and stable anti-angiogenic peptide inhibitors from their parent receptors provides a feasible route to develop novel leads for anti-angiogenic medicines.

L23 ANSWER 8 OF 20 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:92360 The Genuine Article (R) Number: 635ZT. Independent prognostic impact of lymphatic vessel density and presence of low-grade lymphangiogenesis in cutaneous melanoma. Straume O; Jackson D G; Akslen L A (Reprint). Haukeland Univ Hosp, Gade Inst, Dept Pathol, N-5021 Bergen, Norway (Reprint); Univ Bergen, Gade Inst, Dept Pathol, N-5021 Bergen, Norway; John Radcliffe Hosp, Weather Inst Mol Med, MRC, Human Immunol Unit, Oxford OX3 9DS, England. CLINICAL CANCER RESEARCH (JAN 2003) Vol. 9, No. 1, pp. 250-256. Publisher: AMER ASSOC CANCER RESEARCH. PO BOX 11806, BIRMINGHAM, AL 35202 USA. ISSN: 1078-0432. Pub. country: Norway; England. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The aim of this study was to determine lymphatic vessel density (LVD) in a series of nodular melanoma and correlate the findings with the expression of several angiogenic factors, including vascular endothelial growth factor-C, basic fibroblast growth factor (bFGF), patient survival, and clinico-pathologic data. Patients with nodular melanoma and complete follow-up information were included. Lymphatic vessels were immunostained with the LYVE-1 and Podoplanin **antibodies**, and LVD was evaluated in both intra- and peri-tumoral (LVDpt) areas. Median LVD was 6.3 and 12.5 vessels/mm(2) in intra- and peri-tumoral areas, and coexpression of LYVE-1 and Ki-67/MIB-1 in lymphatic endothelial cells within the tumor was demonstrated, indicating active but low-grade lymphangiogenesis. Increased LVDpt was significantly associated with localization on the extremities (P = 0.005), decreased tumor thickness (P = 0.036), absence of vascular invasion (P = 0.004), brisk lymphocytic infiltration (P = 0.018), low proliferative rate by Ki-67 (P = 0.011), increased bFGF expression in tumor cells (P = 0.01) as well as in endothelial cells (P = 0.008), and decreased tumor cell expression of Ephrin-A1 (P = 0.009). Decreased LVD in intra-tumoral areas and LVDpt both predicted improved survival rates in multivariate analyses (for LVDpt, Hazard ratio: 2.1, P = 0.009). We found that decreased LVD was present in thicker and more proliferative tumors (Ki-67) and that increased LVD was significantly associated with improved patient survival in multivariate analysis. In addition, our data suggest the presence of low-grade intra-tumoral lymphangiogenesis in melanoma and a stimulating role of bFGF in lymphangiogenesis.

L23 ANSWER 9 OF 20 MEDLINE on STN

2003312109. PubMed ID: 12839674. Expression of vascular endothelial growth factor (VEGF) C and **VEGF receptor 3** in non-small cell lung cancer. Dong Xin; Qiu Xue-shan; Wang En-hua; Li Qing-chang; Gu Wei. (Department of Pathology, China Medical University, Shenyang 110001, China.) Zhonghua bing li xue za zhi Chinese journal of pathology, (2003 Apr) 32 (2) 128-32. Journal code: 0005331. ISSN: 0529-5807. Pub. country: China. Language: Chinese.

AB OBJECTIVE: To study the relationship between angiogenesis and lymphangiogenesis with the expression of vascular endothelial growth

factor C (VEGF-C) and VEGFR-3 in human non-small cell lung cancer (NSCLC). METHODS: Samples of 76 NSCLC cases with the neighboring noncancerous tissue were studied using anti- VEGF-C, VEGFR-3 and CD34 **antibodies.** Assessment of lymphatic vessel density and microvessel density (MVD) were performed. RESULTS: VEGF-C expression in NSCLC was associating with the differentiation of tumor cells (P = 0.009). Expression of VEGF-C and VEGFR-3 was significantly associated with lymph node metastasis (P = 0.008 and P = 0.013 respectively) and lymphatic invasion (P = 0.027 and P = 0.020 respectively). A significant positive correlation was found between VEGF-C in cancer cells and VEGFR-3 in lymphatic endothelial cells (P = 0.009). The number of lymphatic vessels (P = 0.006) and microvascular (P = 0.046) in VEGF-C positive tumors was significantly larger than in VEGF-C-negative tumors. Lymphatic vessel density was closely related to lymph node metastasis (P = 0.010), lymphatic invasion (P = 0.019) and clinical stages (P = 0.015). MVD was closely related to blood metastasis (P < 0.001) and clinical stages (P < 0.001). Patients with positive VEGF-C expression had a worse prognosis than those with a negative VEGF-C expression (P < 0.001). CONCLUSIONS: VEGF-C/VEGF-D in NSCLCs, are related to lymphangiogenesis and angiogenesis, as well as to the occurrence and the development of lung cancers. VEGF-C promotes intratumoral lymphangiogenesis via VEGFR-3, resulting facilitated invasion of cancer cells into the lymphatic vessels. VEGF-C expression can be a useful predictor of poor prognosis in NSCLC.

L23 ANSWER 10 OF 20 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:530229 Document No.: PREV200300525942. PHOTODYNAMIC THERAPY INDUCES EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND PIGMENT EPITHELIAL DERIVED FACTOR (PEDF). Michels, S. M. [Reprint Author]; Schlotzer-Schrehard, U.; Naumann, G. O. H.; Schmidt-Erfurth, U. [Reprint Author]. Ophthalmology, University of Lubeck, Lubeck, Germany. ARVO Annual Meeting Abstract Search and Program Planner, (2003) Vol. 2003, pp. Abstract No. 2157. cd-rom.
Meeting Info.: Annual Meeting of the Association for Research in Vision and Ophthalmology. Fort Lauderdale, FL, USA. May 04-08, 2003. Association for Research in Vision and Ophthalmology.
Language: English.

AB Purpose:To evaluate the influence of photodynamic therapy (PDT) on the regulation of vascular endothelial growth factor (VEGF), **VEGF receptor 3** (VEGFR-3) and pigment epithelium derived factor (PEDF) in aged eyes. Methods:PDT using verteporfin with the recommended standard parameters was applied to selected areas of the macular region in eyes of elderly patients. Eyes which were scheduled for surgical removal due to untreatable malignancy served as study eyes (n=4). Age-matched donor eyes were used as controls (n=4). Lesions were classified by ophthalmoscopy, fluorescein (FA) and indocyanine green angiography (ICGA) as well as LM/EM histology. Immunohistology using **antibodies** against VEGF, VEGFR-3 and PEDF was performed in PDT-treated areas, untreated collateral areas in study eyes and untreated areas of control eyes. Specimen were fixed in 4% paraformaldehyde/1% glutaraldehyde and embedded in paraffine. 4 mum thick sections were stained using the peroxidase labeled streptavidin-biotin method. Results:All study eyes demonstrated a characteristic hypofluorescence of the treated area by FA/ICGA. Selective damage at the level of choriocapillary endothelial cells was documented by LM/EM histology. VEGF labeling was positive in vascular endothelia of the choriocapillary layer and focally within larger choroidal vessels in treated areas, but was absent in untreated controls. Areas showing VEGF expression also demonstrated upregulation of **VEGF receptor 3** . PEDF expression was present in the retinas of all eyes whether they were treated or not. However, PEDF expression by choroidal endothelial cells was specific for treated areas of study eyes only. Conclusions: PDT using verteporfin induces a characteristic angiogenic response in the retina and choroid of human eyes. Enhanced expression of VEGF, **VEGF receptor 3** and PEDF appears to be

associated with PDT and was documented in normal choroidal endothelial cells following treatment.

L23 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2005 ACS on STN
2002:519260 Document No. 137:211309 Blockade of vascular endothelial growth factor receptor-3 signaling inhibits fibroblast growth factor-2-induced lymphangiogenesis in mouse cornea. Kubo, Hajime; Cao, Renhai; Brakenhielm, Ebba; Makinen, Taija; Cao, Yihai; Alitalo, Kari (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute and Helsinki University Central Hospital, Biomedicum Helsinki, University of Helsinki, Helsinki, 00014, Finland). Proceedings of the National Academy of Sciences of the United States of America, 99(13), 8868-8873 (English) 2002. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB Vascular endothelial growth factor receptor-3 (VEGFR-3) is a major mediator of lymphangiogenesis. Recently, VEGFR-3 ligands, VEGF-C, and VEGF-D were reported to promote tumor lymphangiogenesis and lymphatic metastasis, and these processes were inhibited by blocking of the VEGFR-3-signaling pathway. Here, we have adapted the mouse corneal angiogenesis assay to study potential lymphangiogenic factors and inhibitors. Immunohistochem. anal. with lymphatic endothelial markers showed that VEGF-C induces lymphatic as well as blood vessel growth in the cornea. By contrast, VEGF induced angiogenesis but not lymphangiogenesis. Fibroblast growth factor-2 (FGF-2) stimulated both lymphangiogenesis and angiogenesis. FGF-2 up-regulated VEGF-C expression in vascular endothelial and perivascular cells. Furthermore, administration of blocking anti-VEGFR-3 **antibodies** inhibited the FGF-2-induced lymphangiogenesis. These findings show that VEGFR-3 can mediate lymphangiogenesis induced by other growth factors. Because increased expression of FGF-2 and VEGF-C has been associated with lymphatic metastasis, our results provide a potential strategy for the inhibition of lymphatic metastasis in cancer therapy.

L23 ANSWER 12 OF 20 MEDLINE on STN DUPLICATE 5
2002211556. PubMed ID: 11948478. VEGF-C induced lymphangiogenesis is associated with lymph node metastasis in orthotopic MCF-7 tumors. Mattila Mirjami M-T; Ruohola Johanna K; Karpanen Terhi; Jackson David G; Alitalo Kari; Harkonen Pirkko L. (Department of Anatomy, Institute of Biomedicine and MediCity Research Laboratory, University of Turku, Turku, Finland.) International journal of cancer. Journal international du cancer, (2002 Apr 20) 98 (6) 946-51. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.
AB The spread of cancer cells to regional lymph nodes through the lymphatic system is the first step in the dissemination of breast cancer. In several human cancers including those of the breast and prostate, the expression of vascular endothelial growth factor C (VEGF-C) is associated with lymph node metastasis. Our study was undertaken to evaluate the effect of VEGF-C on metastasis of poorly invasive, estrogen dependent human MCF-7 breast cancer cells. MCF-7 breast cancer cells transfected with VEGF-C (MCF-7-VEGF-C) were grown as tumors in the mammary fat pads of nude mice implanted with subcutaneous estrogen pellets. Tumor lymphangiogenesis and lymph node metastasis were studied immunohistochemically using **antibodies** against lymphatic vessel hyaluronan receptor -1 (LYVE-1), **VEGF receptor-3** (VEGFR-3), PECAM-1, pan-cytokeratin and estrogen dependent pS2 protein. Overexpression of VEGF-C in transfected MCF-7 cells stimulated in vivo tumor growth in xenotransplanted mice without affecting estrogen responsiveness. The resulting tumors metastasized to the regional lymph nodes in 75% (in 6 mice out of 8, Experiment I) and in 62% (in 5 mice out of 8, Experiment II) of mice bearing orthotopic tumors formed by MCF-7-VEGF-C cells whereas no metastases were observed in mice bearing tumors of control vector-transfected MCF-7 cells (MCF-7-Mock). The density of intratumoral and peritumoral lymphatic vessels was increased in tumors derived from MCF-7-VEGF-C cells but not MCF-7-Mock cells. Taken together, our results show that VEGF-C overexpression stimulates tumor

lymphangiogenesis and induces normally poorly metastatic estrogen-dependent MCF-7 tumors to disseminate to local lymph nodes. These data suggest that VEGF-C has an important role in lymph node metastasis of breast cancer even at its hormone-dependent early stage. Copyright 2002 Wiley-Liss, Inc.

L23 ANSWER 13 OF 20 MEDLINE on STN

2002306448. PubMed ID: 12048269. Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. He Yulong; Kozaki Ken-Ichi; Karpanen Terhi; Koshikawa Katsumi; Yla-Herttuala Seppo; Takahashi Takashi; Alitalo Kari. (Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute and Helsinki University Central Hospital, Biomedicum Helsinki, University of Helsinki, Finland.) Journal of the National Cancer Institute, (2002 Jun 5) 94 (11) 819-25. Journal code: 7503089. ISSN: 0027-8874. Pub. country: United States. Language: English.

AB BACKGROUND: Vascular endothelial growth factor C (VEGF-C) stimulates tumor lymphangiogenesis (i.e., formation of lymphatic vessels) and metastasis to regional lymph nodes by interacting with **VEGF receptor 3** (VEGFR-3). We sought to determine whether inhibiting VEGFR-3 signaling, and thus tumor lymphangiogenesis, would inhibit tumor metastasis. METHODS: We used the highly metastatic human lung cancer cell line NCI-H460-LNM35 (LNM35) and its parental line NCI-H460-N15 (N15) with low metastatic capacity. We inserted genes by transfection and established a stable N15 cell line secreting VEGF-C and a LNM35 cell line secreting the soluble fusion protein **VEGF receptor 3**-immunoglobulin (VEGFR-3-Ig, which binds VEGF-C and inhibits VEGFR-3 signaling). Control lines were transfected with mock vectors. Tumor cells were implanted subcutaneously into severe combined immunodeficient mice (n = 6 in each group), and tumors and metastases were examined 6 weeks later. In another approach, recombinant adenoviruses expressing VEGFR-3-Ig (AdR3-Ig) or beta-galactosidase (AdLacZ) were injected intravenously into LNM35 tumor-bearing mice (n = 14 and 7, respectively). RESULTS: LNM35 cells expressed higher levels of VEGF-C RNA and protein than did N15 cells. Xenograft mock vector-transfected LNM35 tumors showed more intratumoral lymphatic vessels (15.3 vessels per grid; 95% confidence interval [CI] = 13.3 to 17.4) and more metastases in draining lymph nodes (12 of 12) than VEGFR-3-Ig-transfected LNM35 tumors (4.1 vessels per grid; 95% CI = 3.4 to 4.7; P<.001, two-sided t test; and four lymph nodes with metastases of 12 lymph nodes examined). Lymph node metastasis was also inhibited in AdR3-Ig-treated mice (AdR3-Ig = 0 of 28 lymph nodes; AdLacZ = 11 of 14 lymph nodes). However, metastasis to the lungs occurred in all mice, suggesting that LNM35 cells can also spread via other mechanisms. N15 tumors overexpressing VEGF-C contained more lymphatic vessels than vector-transfected tumors but did not have increased metastatic ability. CONCLUSIONS: Lymph node metastasis appears to be regulated by additional factors besides VEGF-C. Inhibition of VEGFR-3 signaling can suppress tumor lymphangiogenesis and metastasis to regional lymph nodes but not to lungs.

L23 ANSWER 14 OF 20 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 6

2001:962088 The Genuine Article (R) Number: 497HY. Stimulation of beta(1) integrin induces tyrosine phosphorylation of vascular endothelial growth factor receptor-3 and modulates cell migration. Wang J F; Zhang X F; Groopman J E (Reprint). Harvard Univ, Sch Med, Beth Israel Deaconess Med Ctr, Inst Med, Div Expt Med, 4 Blackfan Circle, Boston, MA 02115 USA (Reprint); Harvard Univ, Sch Med, Beth Israel Deaconess Med Ctr, Inst Med, Div Expt Med, Boston, MA 02115 USA; Harvard Univ, Sch Med, Beth Israel Deaconess Med Ctr, Div Hematol Oncol, Boston, MA 02115 USA. JOURNAL OF BIOLOGICAL CHEMISTRY (9 NOV 2001) Vol. 276, No. 45, pp. 41950-41957. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. ISSN: 0021-9258. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Interactions between integrins and tyrosine kinase receptors can modulate a variety of cell functions. We observed a cooperative interaction between the beta(1) integrin and vascular endothelial growth factor receptor-3 (VEGFR-3 or Flt4) that appeared to be required for cell migration. By using VEGFR-3-transfected 293 cells (293/VEGFR-3) or primary dermal microvascular endothelial cells (DMEC), we found that stimulation with either soluble or immobilized extracellular matrix (ECM) proteins, collagen or fibronectin (FN), resulted in the increased tyrosine phosphorylation of VEGFR-3 in the absence of a cognate ligand. This increased tyrosine phosphorylation of VEGFR-3 was diminished by pretreatment with a blocking **antibody** against the beta(1) integrin. Cross-linking with anti-beta(1) integrin **antibody** induced a similar degree of tyrosine phosphorylation of VEGFR-3. Stimulation with collagen or FN induced an association between beta(1) integrin and VEGFR-3 in both 293/VEGFR-3 and primary DMEC cells. Collagen or FN-induced tyrosine phosphorylation of VEGFR-3 was inhibited by treatment with cytochalasin D, an inhibitor of actin polymerization. Collagen or FN was able to induce the migration of 293/VEGFR-3 or DMEC cells to a limited extent. However, migration was dramatically enhanced when a gradient of the cognate ligand, VEGF-D, was added. VEGF-D failed to induce cell migration in the absence of ECM proteins. Introducing a mutation at the kinase domain of VEGFR-3 or treatment with blocking **antibody** against either VEGFR-3 or beta(1) integrin inhibited cell migration induced by ECM and VEGF-D, indicating that signals from both beta(1) integrin and VEGFR-3 are required for this cell function.

L23 ANSWER 15 OF 20 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:287339 Document No.: PREV200100287339. Lymphatic capillaries in vascularized human corneas: Ultrastructural and immunohistochemical evidence using **VEGF receptor** 3/flt-4 and podoplanin as specific markers. Cursiefen, C. [Reprint author]; Schloetzer-Schrehardt, U. [Reprint author]; Breiteneder-Geleff, S.; Alitalo, K.; Kuechle, M. [Reprint author]; Naumann, G. O. [Reprint author]. Augenklinik mit Poliklinik, Univ of Erlangen - Nuernberg, Erlangen, Germany. IOVS, (March 15, 2001) Vol. 42, No. 4, pp. S123. print. Meeting Info.: Annual Meeting of the Association for Research in Vision and Ophthalmology. Fort Lauderdale, Florida, USA. April 29-May 04, 2001. Language: English.

L23 ANSWER 16 OF 20 MEDLINE on STN DUPLICATE 7

2000247148. PubMed ID: 10785369. Monoclonal **antibodies** to vascular endothelial growth factor-D block its interactions with both VEGF receptor-2 and **VEGF receptor-3.** Achen M G; Roufail S; Domagala T; Catimel B; Nice E C; Geleick D M; Murphy R; Scott A M; Caesar C; Makinen T; Alitalo K; Stacker S A. (Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Victoria, Australia.. marc.achen@ludwig.edu.au) . European journal of biochemistry / FEBS, (2000 May) 267 (9) 2505-15. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Vascular endothelial growth factor-D (VEGF-D), the most recently discovered mammalian member of the VEGF family, is an angiogenic protein that activates VEGF receptor-2 (VEGFR-2/Flk1/KDR) and VEGFR-3 (Flt4). These receptor tyrosine kinases, localized on vascular and lymphatic endothelial cells, signal for angiogenesis and lymphangiogenesis. VEGF-D consists of a central receptor-binding VEGF homology domain (VHD) and N-terminal and C-terminal propeptides that are cleaved from the VHD to generate a mature, bioactive form consisting of dimers of the VHD. Here we report characterization of mAbs raised to the VHD of human VEGF-D in order to generate VEGF-D antagonists. The mAbs bind the fully processed VHD with high affinity and also bind unprocessed VEGF-D. We demonstrate, using bioassays for the binding and cross-linking of VEGFR-2 and VEGFR-3 and biosensor analysis with immobilized receptors, that one of the mAbs, designated VD1, is able to compete potently with mature VEGF-D for binding to both VEGFR-2 and VEGFR-3 for binding to mature VEGF-D. This indicates

that the binding epitopes on VEGF-D for these two receptors may be in close proximity. Furthermore, VD1 blocks the mitogenic response of human microvascular endothelial cells to VEGF-D. The anti-(VEGF-D) mAbs raised to the bioactive region of this growth factor will be powerful tools for analysis of the biological functions of VEGF-D.

L23 ANSWER 17 OF 20 MEDLINE on STN DUPLICATE 8

2000391496. PubMed ID: 10887117. Involvement of vascular endothelial growth factor receptor-3 in maintenance of integrity of endothelial cell lining during tumor angiogenesis. Kubo H; Fujiwara T; Jussila L; Hashi H; Ogawa M; Shimizu K; Awane M; Sakai Y; Takabayashi A; Alitalo K; Yamaoka Y; Nishikawa S I. (Departments of Gastroenterological Surgery and Molecular Genetics, Graduate School of Medicine, Kyoto University, Kyoto, Japan.. kubofit@kuhp.kyoto-u.ac.jp) . Blood, (2000 Jul 15) 96 (2) 546-53. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) plays a major role in tumor angiogenesis. VEGF-C, however, is thought to stimulate the growth of lymphatic vessels because an expression of its specific receptor, **VEGF receptor-3** (VEGFR-3), was demonstrated to be restricted to lymphatic vessels. Here we demonstrate that the inactivation of VEGFR-3 by a novel blocking monoclonal **antibody** (mAb) suppresses tumor growth by inhibiting the neo-angiogenesis of tumor-bearing tissues. Although VEGFR-3 is not expressed in adult blood vessels, it is induced in vascular endothelial cells of the tumor-bearing tissues. Hence, VEGFR-3 is another receptor tyrosine kinase involved in tumor-induced angiogenesis. Micro-hemorrhage in the tumor-bearing tissue was the most conspicuous histologic finding specific to AFL4 mAb-treated mice. Scanning microscopy demonstrated disruptions of the endothelial lining of the postcapillary venule, probably the cause of micro-hemorrhage and the subsequent collapse of the proximal vessels. These findings suggest the involvement of VEGFR-3 in maintaining the integrity of the endothelial lining during angiogenesis. Moreover, our results suggest that the VEGF-C/VEGFR-3 pathway may serve another candidate target for cancer therapy. (Blood. 2000;96:546-553)

L23 ANSWER 18 OF 20 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:312317 Document No.: PREV200100312317. Stimulation of beta-1 integrin induces tyrosine phosphorylation of **VEGF receptor-3** and modulates cell migration in endothelial cells. Wang, J.-F. [Reprint author]; Zhang, X.-F. [Reprint author]; Groopman, J. E. [Reprint author]. Divisions of Experimental Medicine and Hematology/Oncology, Beth Israel Deaconess Medical Center, HMS, Boston, MA, USA. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 530a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Cooperation and interaction between integrins and tyrosine kinase receptors act to regulate endothelial cell proliferation, differentiation, survival, and migration. We have observed cooperative interaction between the beta-1 integrin and **VEGF receptor-3** (VEGFR-3 or Flt4), and this interaction appears to be required for cell migration. Using VEGFR-3-transfected 293 cells (293/VEGFR-3) or primary dermal microvascular endothelial cells (DMEC), we demonstrated that stimulation with either soluble or immobilized extracellular matrix (ECM) protein, collagen, fibronectin or laminin, resulted in the increased tyrosine phosphorylation of VEGFR-3 in the absence of receptor ligands. This increased tyrosine phosphorylation was diminished by pretreatment with a blocking **antibody** against beta-1 integrin. Cross-linking with anti-beta-1 integrin **antibody** induced a similar degree of tyrosine phosphorylation of VEGFR-3 in both (293/VEGFR-3) and DMEC. A constitutive association between beta-1 integrin and VEGFR-3 was observed by co-immunoprecipitation analysis, and this association was enhanced by

the stimulation with either ligand for beta integrin or VEGFR-3. We further found that collagen or fibronectin was able to induce migration of 293/VEGFR-3 or DMEC to a limited extent; however, migration was significantly enhanced when a VEGF-D gradient was added. VEGF-D failed to induce cell migration in the absence of matrix proteins. Cell migration induced by matrix proteins and/or VEGF-D was inhibited by blocking **antibody** against either VEGFR-3 or beta-1 integrin. The tyrosine phosphorylation of VEGFR-3 induced by beta-1 integrin engagement, and the cell migration induced by VEGF-D, were each inhibited by pretreatment with GF109203X, a PKC inhibitor, or cytochalasin D, an inhibitor of actin polymerization. Our results suggest that the stimulation of beta-1 integrin cross-activates VEGFR-3 and that this receptor-receptor interaction is required in the process of endothelial cell migration.

L23 ANSWER 19 OF 20 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

1999:958648 The Genuine Article (R) Number: 263KK. Lack of lymphatic vascular specificity of vascular endothelial growth factor receptor 3 in 185 vascular tumors. Partanen T A; Alitalo K; Miettinen M (Reprint). ARMED FORCES INST PATHOL, DEPT SOFT TISSUE PATHOL, 14TH ST & ALASKA AVE NW, WASHINGTON, DC 20306 (Reprint); ARMED FORCES INST PATHOL, DEPT SOFT TISSUE PATHOL, WASHINGTON, DC 20306; UNIV HELSINKI, HAARTMAN INST, MOL CANC BIOL LAB, HELSINKI, FINLAND. CANCER (1 DEC 1999) Vol. 86, No. 11, pp. 2406-2412 . Publisher: WILEY-LISS. DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012. ISSN: 0008-543X. Pub. country: USA; FINLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB BACKGROUND. Among the molecules important to angiogenesis and lymphangiogenesis is vascular endothelial growth factor receptor 3 (VEGFR-3), a member of the receptor tyrosine kinases of endothelial cells. This receptor is expressed consistently in normal lymphatics, lymphangiomas, and in Kaposi sarcoma; but data regarding other vascular tumors are scant.

METHODS. In this study the authors immunohistochemically examined VEGFR-3 expression in 82 benign, 31 borderline, and 72 malignant vascular tumors using a monoclonal **antibody** to VEGFR-3, heat-induced epitope retrieval, and an avidin-biotin-peroxidase detection system.

RESULTS. Although normal mesenchymal tissues showed VEGFR-3 only in the lymphatics, benign and malignant vascular tumors and neovascularization of nonendothelial tumors showed widespread VEGFR-3 distribution. All lymphangiomas and Kaposi sarcomas showed consistent VEGFR-3 reactivity. Among the hemangiomas, spindle cell hemangiomas and 80% of capillary (including all lobular capillary hemangiomas) were positive whereas the endothelium of cavernous, venous, and epitheloid hemangiomas were positive in a minority of cases (20%, 27%, and 33%, respectively). Among the borderline lesions, Kaposiform hemangioendotheliomas were intensely positive whereas epithelioid hemangioendotheliomas were positive in 11 of 29 cases (38%). Angiosarcomas showed VEGRF-3 reactivity in the majority of cases (48 of 60 cases; 80%). The nonepithelioid variants more often were positive (40 of 45 cases; 89%) than the epithelioid variants, of which 8 of 15 (53%) showed positive tumor cells. Nonvascular tumors (including perivascular tumors, other sarcomas, melanomas, carcinomas, and large cell lymphomas) consistently were negative whereas tumor neovascularization commonly was VEGFR-3 positive.

CONCLUSIONS. The results of the current study show that although VEGFR-3 shows specificity toward lymphatics in normal tissues, this receptor is distributed extensively in benign and malignant vascular tumors and therefore can be considered a novel marker in the assessment of endothelial cell differentiation of Vascular neoplasms. (C) 1999 American Cancer Society.

L23 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2005 ACS on STN

2000:275740 Document No. 133:175463 The expression of vascular endothelial growth factor type C and its receptors, and their clinical implications in primary non-small cell lung cancers--the association with lymphatic

metastasis. Kajita, Takeshi (Department of Surgery (I), School of Medicine, Kanazawa University, Kanazawa, 920-8640, Japan). Kanazawa Daigaku Juzen Igakkai Zasshi, 108(6), 695-707 (Japanese) 1999. CODEN: JUZIAG. ISSN: 0022-7226. Publisher: Juzen Igakkai.

AB To evaluate the role of vascular endothelial growth factor type C (VEGF-C) in primary non-small cell lung cancer, VEGF-C mRNA and protein expression were analyzed using 6 lung cancer cell lines, 30 freshly frozen resected specimens, and 62 paraffin-embedded specimens. For the assessment of the correlation between VEGF-C and its receptors, **VEGF receptor-3** (VEGFR-3) and VEGF receptor-2 (VEGFR-2) expression were also analyzed. Reverse transcription PCR anal. revealed that VEGF-C mRNA was detected in 66.6% (4/6) of lung cancer cell lines examined In clin. samples, over-expression of VEGF-C mRNA in the tumor tissue as compared to the adjacent normal lung tissue was not clear. In three of six (50%) lung cancer cell lines, VEGFR-3 mRNA was detected. Western blot anal. revealed that VEGFR-3 protein was detected in five of six (83.3%) lung cancer cell lines. As a result of immunohistochem., the VEGF-C antigen was mainly observed in the cytoplasm of cancer cells. The percentage of the patients with pos. VEGF-C expression was 38.7% (24/62). A significant pos. correlation was clearly found between the VEGF-C expression in cancer cells and VEGFR-3 expression in vascular endothelial cells ($p<0.01$). Moreover, the expression of VEGF-C protein in cancer cells was significantly associated with the intensity of both VEGFR-3 and VEGFR-2 staining in cancer cells (both $p<0.05$). The relationship between microvessel d. evaluated by using **antibody** against von Willebrand factor and VEGF-C protein expression was not statistically significant. The VEGF-C protein expression was significantly greater in the male group (46.6%) than in the female group (17.6%). The VEGF-C protein expression in patients with lymph node metastasis (56.0%) was significantly greater than that in patients without lymph node metastasis (27.0%) ($p<0.05$). The VEGF-C protein expression in patients with pos. lymphatic invasion (58.3%) was significantly greater than that in patients with neg. lymphatic invasion (26.3%) ($p<0.05$). The VEGF-C protein expression in patients with stage III/IV Diseases (55.5%) was significantly greater than that in those with stage I/II diseases (25.7%) ($p<0.05$). Univariate anal. demonstrated that the patients with VEGF-C protein expression had significantly poorer outcomes as compared to those without it ($p<0.05$). These results suggest that VEGF-C is associated with lymphatic metastasis and tumor progression. As for the mechanisms underlying the lymphatic metastasis, the results of this study suggested the existence of an autocrine mechanism that may induce some biol. behavior in lung cancer cells and promote cancer development, in addition to the paracrine mechanism that facilitates angiogenesis/lymphangiogenesis.

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